Bayer HealthCare
Pharmaceuticals

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MAGNEVIST® (brand of gadopentetate dimeglumine)
Injection and Pharmacy Bulk Package

RE: **Sponsors Background Package for FDA Advisory Committee Meeting – December 8, 2009**

November 3, 2009
Bayer HealthCare
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Reference is made to a written correspondence from the Office of Executive Programs, Division of Advisory Committee and Consultants Management, dated August 18, 2009. In this communication the Division was informing Bayer HealthCare Pharmaceuticals of a joint advisory committee meeting of the Cardiovascular and Renal Drugs and the Drug Safety and Risk Management Advisory Committee. The topic of this meeting was identified to be gadolinium based contrast agents.

Reference is also made to subsequent electronic correspondences, telephone conversations and requested submissions in preparation of this advisory committee meeting scheduled to take place on December 8, 2009 in Gaithersburg, Maryland.

Bayer HealthCare Pharmaceuticals is pleased to participate in this important medical communication and has enclosed, in this submission, a detailed briefing package for Magnevist; summarizing the possible risk factors of Magnevist in relation to the development of Nephrogenic Systemic Fibrosis (NSF). Please find the following documentation and related references:

Title: ***Overall risk assessment of Magnevist and other GBCAs in the context of Nephrogenic Systemic Fibrosis (NSF): Summary of possible risk factors and currently available scientific data***

Authors: S Dohanish, H Goldstein, E Köfüncü, A Michel, C Pering, H Pietsch, M Schultze-Mosgau, and T Steger-Hartmann

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If you have any additional questions or need additional information, please contact me, Michele DeBartolo MPH, RD., at 973-294-8153 or, in my absence, John Talian at 973-487-2789. For technical support or questions, please contact Shelley Drost at 973-487-2474.

Sincerely,

Signed by Kevin Hibbert, MD for
Michele DeBartolo, MPH RD
Independent Consultant .
Global Regulatory Affairs

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List of abbreviations

ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AST	Aspartate Transaminase
AUC	Overall Systemic Exposure [Area Under the Curve]
BSP	Bayer Schering Pharma
BW	Body Weight
Ca	Calcium
$CaCl_2$	Calcium Chloride
Ca-EDTA	Calcium ethylenediamine tetraacetic acid
CE-MRI	Contrast enhanced Magnetic Resonance Imaging
CKD	Chronic Kidney Disease
CLt	Total Serum Clearance
CMD	Compartment-Model Dependent
CMID	Compartment-Model Independent
CNS	Central nervous system
CRF	Case Report Forms
CT	Computed tomography
Cu	Copper
DNA	Deoxyribonucleic Acid
DTPA	Diethylene triamine pentaacetic acid
ED1-1	Macrophages
EDX	Electron Dispersive X-ray
eg	for example
eGFR	Estimated glomerular filtration rate
ESRD	End-Stage Renal Disease
ESRF	End-Stage Renal Failure
FDA	Food and Drug Administration
GBCA	Gadolinium Based Contrast Agents
Gd	Gadolinium
Gd-DTPA	Gadolinium diethyltriamine pentaacetic acid
Gd-EDTA	Gadolinium ethylenediamine tetraacetic acid
GGT	Gamma Glutamyl Tranferase
GLP	Good Laboratory Practices
h	Hour
ICP-AES	Inductive-Coupled Plasma – Atomic Emission Spectroscopy
ICP-OES	Inductive-Coupled Plasma – Optical Emission Spectrometry
ie	that is
IgA	Immunglobuline A
IgE	Immunglobuline E
i.v.	Intravenous
Ktherm	Thermodynamic Stability
MedDRA	Medical Dictionary of Regulatory Activities

MCP	Monocyte Chemoattractant Proteins
Mg	Magnesium
MIP	Macrophage Inflammatory Proteins
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NDA	New Drug Application
NFD	Nephrogenic Fibrosing Dermopathy
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed Adverse Effect Level
NO-precursor	Nitrogen oxide precursor
NSF	Nephrogenic Systemic Fibrosis
PCA	Passive Cutaneous Anaphylaxis
p.c.	Post conception
p.i.	Post-injection
p.o.	Per oral
p.p.	Post partum
PWG	Pathology Working Group
RBC	Erythrocytes [Red Blood Cells]
RES	Reticulo-endothelial system
SAE	Serious Adverse Events
s.c.	Subcutaneous
SD	Standard Deviation
SOC	System Organ Class
SPIOs	Superparamagnetic Iron Oxide Particles
T1	Longitudinal Relaxation Time
t 1/2	Half Life
T2	Transversal Relaxation Time
TNF-α	Tumor Necrosis Factor-α
US	United States
VEGF	Vascular Endothelial Growth Factor
VSS	Volume of distribution at steady state
Zn	Zinc

1. Overview of contrast-enhanced magnetic resonance imaging – clinical use and diagnostic benefit

Magnetic Resonance Imaging (MRI) is an important medical imaging technique for diagnosing and monitoring disease. MRI permits visualization of structure and function. It provides better differentiation of soft tissue than computed tomography (CT), and unlike CT, it does not use ionizing radiation.

For a number of diseases of the brain and spine, MRI is the primary method of imaging. MRI of the brain or spine has been shown to be effective when searching for known or suspected primary or metastatic tumors, infection, inflammation, demylinating disease, trauma, or degenerative disease. While not as frequent as MRI examinations of the central nervous system (CNS), MRI also has a major role in examining other parts of the body. The role of MRI in examining organs of the chest, abdomen and pelvis is well recognized. For example, MRI of the body can be used to diagnose tumors, diseases of the liver, cysts of the kidneys, fibroids and endometriosis.

Magnetic resonance (MR) contrast agents are used to increase the diagnostic capabilities of the MRI examination. MR contrast agents have been shown to improve lesion conspicuity as well as visualization. Furthermore, MR contrast agents have been shown to improve detection of the number on lesions, lesion borders, lesion size, and lesion location. MR contrast agents may also provide information helpful to characterize lesions. MR enhancement, as well as the absence of enhancement, may aid in the interpretation of an MRI.

MR contrast agents have been available for more than two decades. The majority of MR contrast agents are based on Gadolinium (Gd), a rare earth metal with paramagnetic properties. As such, gadolinium (Gd) based contrast agents (GBCA) act by influencing the local magnetic field of nearby water molecules when placed in a magnetic field. The high magnetic moment produced by the paramagnetic agents result in a relatively large local magnetic field, which can enhance the relaxation rates of water protons in the vicinity of the paramagnetic agent.

In MRI, visualization of normal and pathological tissue depends in part on variations in the radiofrequency signal intensity that occur with longitudinal relaxation time (Tl) and transverse relaxation time (T2).

MRI is a frequently used diagnostic test. It is estimated that there were approximately 35 million MRIs performed in the United States (US) in 2008 and that approximately 28% of these MRIs were contrast enhanced. A possible association between Nephrogenic Systemic Fibrosis (NSF) and GBCAs was first suggested in 2006.

Magnevist (INN: gadopentetate dimeglumine) Injection was the first gadolinium-based MR contrast agent to be approved in the US for use with MRI in1988. It has the broadest range of indications of GBCAs in adult and pediatric patients in the US. More than a total of 100 million doses of Magnevist have been administered worldwide to date with nearly half of those in the US.

For an overview of all GBCAs and their active ingredients see[1]

2. Magnevist product overview

2.1 Magnevist-enhanced MRI

Magnevist (Gadopentetate, INN: gadopentetate dimeglumine) was approved in 1988 in Europe, the United States and Japan. It was the first Gadolinium-based contrast agent (GBCA) approved for clinical use in magnetic resonance imaging (MRI). Magnevist is currently marketed in over 100 countries.

More than a total of 100 million doses of Magnevist have been administered worldwide. This is approximately twice the number of administrations of the next most frequently administered GBCA (Omniscan). Approximately, 49 million of these Magnevist doses have been administered in the US. Since Gadolinium contrast-enhanced MRl were first introduced into clinical medicine in the 1980’s, it is estimated that more than half of all of these

procedures have been performed with Magnevist. The most frequent GBCA contrast-enhanced MRI examination is that of the central nervous system (CNS), ie, brain or spine.

Magnevist is a paramagnetic agent and, as such, it develops a magnetic moment when placed in a magnetic field. The high magnetic moment produced by the paramagnetic agent results in a relatively large local magnetic field, which can enhance the relaxation rates of water protons in the vicinity of the paramagnetic agent.

In MRI, visualization of normal and pathological tissue depends in part on variations in the radiofrequency signal intensity that occur with longitudinal relaxation time (Tl) and transverse relaxation time (T2). When placed in a magnetic field, Magnevist decreases the Tl and T2 relaxation time in tissues where it accumulates. At clinical doses the effect is primarily on the T1 relaxation time.

After intravenous bolus injection Magnevist rapidly distributes into the extracellular space. Magnevist does not cross the intact blood-brain barrier and, therefore, does not accumulate in normal brain or in lesions with an intact blood-brain barrier, e.g., cysts, mature post-operative scars, etc. However, disruption of the blood-brain barrier or abnormal vascularity allows for the accumulation of Magnevist in lesions such as neoplasms, abscesses, and subacute infarcts.

In the US, Magnevist has the broadest range of indications of all marketed GBCAs and is the only GBCA that is approved and indicated for all of the following types of MR imaging - CNS, extracranial-extraspinal tissues (head, neck) and body (excluding the heart) in both adult and pediatric populations from 2 years onwards. The indications are detailed in the following paragraphs:

Central Nervous System

Magnevist is indicated for use with magnetic resonance imaging (MRI) to visualize lesions with abnormal vascularity in the brain (intracranial lesions), spine and associated tissues. Magnevist has been shown to facilitate visualization of intracranial lesions including but not limited to tumors.

Extracranial Extraspinal Tissues

Magnevist is indicated for use with MRI in adults and pediatric patients (2 years of age and older) to facilitate the visualization of lesions with abnormal vascularity in the head (extracranial) and neck.

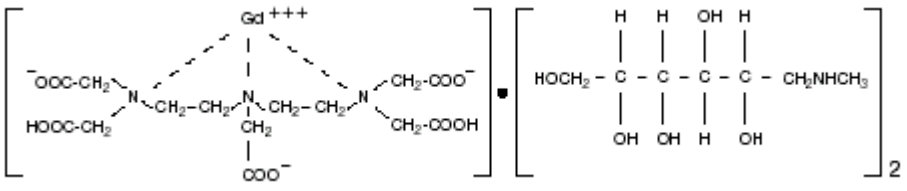
Body

Magnevist is indicated for use in MRI to facilitate the visualization of lesions with abnormal vascularity in the body (excluding the heart).

The recommended dosage of Magnevist approved in the US is 0.2 mL/kg (0.1 mmol Gd/kg body weight [BW]) administered intravenously, at a rate not to exceed 10 mL per 15 seconds. In other countries dosages up to 0.6 mL/kg (0.3 mmol Gd/kg body weight) may be administered as a single intravenous bolus injection.

2.2 Physicochemical properties

Magnevist is the N-methylglucamine salt of the gadolinium complex of diethylenetriamine pent acetic acid and is provided as a 0.5-mol/L solution. Each mL of Magnevist contains 469.01 mg gadopentetate dimeglumine, 0.99 mg meglumine, 0.40 mg diethylenetriamine pent acetic acid and water for injection. Magnevist contains no antimicrobial preservative.



Text Figure 1: Magnevist

Magnevist belongs to the group of linear (open-chain) chelates, which are characterized by thermodynamic (log K therm, valid at pH 14) and conditional complex stability (log K cond, calculated for pH 7.4 with use of the protonation constants of the ligand).

Text Table 1: Complex stability of Magnevist

Charge	Thermodynamic stability (log K therms pH 14	Conditional stability (log K conds pH 7.4)
Ionic	22.5	18.4

Magnevist has an osmolality 6.9 times that of plasma (285 mOsmol/kg). Magnevist is hypertonic under conditions of use. At the standard dose of 0.1 mmol/kg of Magnevist, the total osmotic load for a 70 kg patient would be 27 mOsm.

2.3 Pharmacokinetics

The pharmacokinetics of intravenously administered gadopentetate, the active ingredient of Magnevist, in healthy subjects conform to a two compartment open-model with mean distribution and elimination half-lives (reported as mean ± standard deviation [SD]) of about 0.2 ± 0.13 hours and 1.6 ± 0.13 hours, respectively. Gadopentetate is predominantly eliminated via the kidneys with 83 ± 14% (mean ± SD) of the dose excreted within 6 hours and 91 ± 13% (mean ± SD) by 24 hours post-injection (p.i.). There is neither biotransformation nor decomposition of gadopentetate dimeglumine.

The renal and plasma clearance rates (1.76 ± 0.39 mL/min/kg and 1.94 ± 0.28 mL/min/kg, respectively) of gadopentetate are essentially identical, indicating an absence of nonrenal routes. The volume of distribution (266 ± 43 mL/kg) is equal to extracellular water and the clearance is similar to glomerular filtration.

In vitro results indicate that gadopentetate does not bind to human plasma protein.

Gadopentetate can be removed from the body by hemodialysis.

2.4 Summary of clinical safety

Magnevist has demonstrated a favorable efficacy and safety profile both in clinical trials and during the post-marketing surveillance period.

In 1272 patients in clinical trials, upon which the US New Drug Application (NDA) approval was based, the most common adverse reaction was headache (4.8%). Other adverse drug reactions that occurred in ≥1% of the patients included: nausea (2.7%), injection site coldness/localized coldness (2.3%) and dizziness (1%). Additional reactions that occurred in less than 1% of patients are included in the US Package Insert[2].

Between 1985 and 2005, more than 11,000 subjects were enrolled globally (incorporating the data from the US clinical trials mentioned above) in clinical Phase I to Phase III studies, in which Magnevist was investigated for use in various indications, at varying doses, in various patient populations, and in different countries. Adverse event rates were consistently low, with no event reaching a frequency greater than "uncommon" (≥ 1/1000 to < 1/100).

In a post-marketing safety study in 15,496 patients conducted in 1992[3], adverse drug reactions were reported in 2.4% (n = 372) of patients. Most reactions were minor and transient, with nausea and headache occurring most frequently. Only two serious reactions occurred, both of which were attributed to underlying disease.

In a 2006 publication based on data through 2003 - after 15 years of use and 45 million administrations of Magnevist - a worldwide assessment of utilization was conducted to review the overall safety, risk, and extended clinical experience with Magnevist[4]. The overall reporting rate of spontaneous adverse events was 0.018%.

An additional review of the worldwide clinical experience with Magnevist was conducted after more than 69 million administrations[5]. The review includes that as of Dec 31, 2005, a total of 13,439 patients had participated in protocolled Phase IIIb-IV studies in Europe, during which 198 patients (1.47%) reported 239 adverse events. Additionally, spontaneous adverse events were reported to and collected by the manufacturer for 11,536 patients, a reporting rate of 0.02% of the estimated number of applications. The report noted that during

the past few years, a nephrotoxic potential of the gadolinium-based contrast agents in patients with pre-existing renal impairment had been recognized. Generally associated with the administration of higher doses (eg, > 0.3 mmol/kg), rare cases of acute renal failure and increased creatinine had been reported in association with Magnevist (reporting rate less than one case per million patients exposed).

2.4.1 Adverse drug reactions from clinical trials

Based on experience in more than 11,000 patients, the following undesirable effects have been observed and classified by investigators in clinical trials with Magnevist as possibly drug-related.

Text Table 2 lists the adverse drug reactions reported in clinical trials of more than 11,000 patients by MedDRA System Organ Classes (MedDRA SOCs).

Text Table 2: Adverse drug reactions reported in clinical trials following administration of Magnevist

System Organ Class	Uncommon (≥1/1,000 to <1/100)	Rare (<1/1,000)
Nervous system disorders	Dizziness Headache Dysgeusia	Convulsion Disorientation Paresthesia Burning sensation Tremor
Eye disorders		Conjunctivitis
Cardiac disorders		Tachycardia
Vascular disorders		Thrombophlebitis Flushing Vasodilatation
Respiratory, thoracic and mediastinal disorders		Dyspnea Throat irritation / Throat tightness Pharyngolaryngeal pain/ Pharynx discomfort Cough Sneezing Wheezing
Gastrointestinal disorders	Vomiting Nausea	Abdominal pain Stomach discomfort Diarrhea Toothache Dry mouth Oral soft tissue pain and paresthesia Salivary hypersecretion

continued

Text Table 2: Adverse drug reactions reported in clinical trials following administration of Magnevist (continued)		
System Organ Class	**Uncommon** **(≥1/1,000 to <1/100)**	**Rare** **(<1/1,000)**
Skin and subcutaneous tissue disorders		Urticaria Pruritus Rash Erythema
Musculoskeletal disorders		Pain in extremity
General disorders and administration site conditions	Pain Feeling hot Feeling cold Various kinds of Injection site reactions*	Edema face Chest pain Pyrexia Edema peripheral Malaise Fatigue Thirst

** Various kinds of Injection site reactions (Injection site coldness, Injection site paresthesia, Injection site swelling, Injection site warmth, Injection site pain, Injection site edema, Injection site irritation, Injection site hemorrhage, Injection site erythema, Injection site discomfort).*

2.4.2 Adverse drug reactions from post-marketing surveillance data

Additional adverse events observed during the post marketing surveillance period are listed in Text Table 3 in MedDRA SOCs.

Text Table 3: Adverse drug reactions reported during post-marketing surveillance following administration of Magnevist

System Organ Class	Rare (< 1/1,000)
Blood and lymphatic system disorders	Iron serum increased
Immune system disorders	Anaphylactoid shock/ Anaphylactoid reactions Hypersensitivity reactions
Psychiatric disorders	Agitation Confusion
Nervous system disorders	Coma Loss of consciousness Somnolence Speech disorder Parosmia
Eye disorders	Visual disturbance Eye pain Lacrimation
Ear and labyrinth disorders	Hearing impaired Ear pain
Cardiac disorders	Cardiac arrest Heart rate decreased Reflex tachycardia
Vascular disorders	Shock Syncope Vasovagal reaction Hypotension Blood pressure increased

continued

Text Table 3: Adverse drug reactions reported during post-marketing surveillance following administration of Magnevist (continued)	
System Organ Class	**Rare** **(< 1/1,000)**
Respiratory, thoracic and mediastinal disorders	Respiratory arrest Respiratory distress Respiratory rate increased or Respiratory rate decreased Bronchospasm Laryngospasm Laryngeal edema Pharyngeal edema Pulmonary edema Cyanosis Rhinitis
Gastrointestinal disorders	Salivation
Hepatobiliary disorders	Blood bilirubin increased Hepatic enzyme increased
Skin and subcutaneous tissue disorders	Angioedema
Musculoskeletal and connective tissue disorders	Back pain Arthralgia
Renal and urinary disorders	Acute renal failure* Increased serum creatinine* Urinary incontinence Urinary urgency
General disorders and administration site conditions	Chills Sweating Body temperature increased or Body temperature decreased Various kinds of injection site reactions**

* *In patients with preexisting renal impairment*

** *Various kinds of injection site reactions (Injection site necrosis, injection site thrombophlebitis, injection site phlebitis, injection site inflammation, injection site extravasation)*

Additionally, in patients with dialysis-dependent renal failure who received Magnevist, delayed and transient inflammatory-like reactions such as fever, chills and C-reactive protein increase have been commonly (> 1%) observed. These patients had MRI examination with Magnevist on the day before hemodialysis.

As discussed in detail below , since a potential association between the administration of GBCAs and development of a condition now known as nephrogenic systemic fibrosis (NSF) was first suggested in early 2006, there have been reports of patients who, according to the report, developed NSF following Magnevist administration.

Based on sales figures, the estimated postmarketing patient exposure until 30 Sep 2009 was over 100,000,000 patients.

2.5 Summary of nonclinical safety

2.5.1 Overview of Magnevist toxicology data from animal studies conducted by BSP (Product's development program)

The toxicological characterization of Magnevist (Gadopentetate; INN: gadopentetate dimeglumine) was designed in concordance with international guidelines to support the risk assessment of a single bolus i.v. (1 mL/kg) administration of Magnevist to humans at a dose of 0.10 to 0.30 mmol/kg (3.7 to 11.1 mmol/m^2).

In particular, the following studies were performed:

- **Acute toxicity** was assessed in single dose intravenous toxicity studies in mice, rats, dogs, and rabbits. Additionally an expanded single dose toxicity study was performed in Cynomolgus monkeys.

- **Repeated dose toxicity** intravenous studies were performed in rats and Beagle dogs with dosing over 4 to 5 weeks.

- A standard battery of tests suitable for the detection of gene, chromosome and genome mutations was performed to evaluate the **mutagenicity** of gadopentetate dimeglumine. In particular, reverse mutation assays were conducted in bacterial and eukaryotic cell systems. Elicitation of deoxyribonucleic acid (DNA) repair was investigated in rat hepatocytes and cellular transformation in mouse embryo fibroblasts. Furthermore the potential genotoxic effects of gadopentetate dimeglumine were assessed in vivo in a dominant-lethal test and in a micronucleus tests in mice and Beagle dogs.

- In order to assess the effects of gadopentetate dimeglumine on reproduction, a complete set of **reproduction toxicology** studies were performed to cover all stages of the reproduction cycle. This program comprised fertility studies with an intraperitoneal and intravenous route in rats, embryotoxicity studies with intravenous administration in rats and rabbits, and a peri-/ postnatal study (intravenous) in rats.

- **Local tolerance** studies were performed after intravenous, intra-arterial, intramuscular, intraperitoneal, paravenous, paravasal, conjunctival, subcutaneous, or intrapulmonary application.

- The program also included studies on the **contact sensitization and antigenicity potential**.

- Due to recent clinical concerns about the potential of GBCAs to induce NSF in some patients, a re-read of the histological preparations of the skin from 4 week studies conducted in the 1980s was performed in 2007 by different pathologists. The aim of this peer review was to ascertain the original findings, that no effects on the skin were detected in the years of study conduct by the respective study pathologists.

All studies pivotal for risk assessment were performed according to GLP regulations.

Bayer recently performed orientating studies with single intravenous administration of Magnevist, Eovist and Gadovist to neonate rats (administration: day 4 post partum).

Preliminary results from the orientating Gadovist study point towards the general feasibility of the chosen model. In addition, no findings were observed which would point to new target organs or effects previously unknown from the existing systemic toxicity studies after single or repeated administration to adult rats.

Based on these results Bayer is currently performing a pivotal extended single dose study with Gadovist (results expected for end of 2009) and plans to conduct similar studies with Magnevist and Eovist in 2010. Furthermore, Bayer is currently evaluating the most recent request from FDA (dated 26.10.09) with regard to the conduct of a repeated dose toxicity study in neonate rats.

2.5.2 Results of Magnevist toxicological studies

In all species in which death occurred after single intravenous administration of high doses of gadopentetate dimeglumine, mortality occurred during or shortly after injection of the test material. The maximum dose volume of gadopentetate dimeglumine given intravenously to mice was 20 mL/kg (delivering 10 mmol/kg or 30 mmol/m²) and the maximum volume given to rats was 30 mL/kg (delivering 15 mmol/kg of 90 mmol/m²). The minimum lethal dose levels observed in adult rodents were 4.8 mmol/kg (14.4 mmol/m²) and 5.3 mmol/kg (15.9 mmol/m²) in male and female mice, respectively, and 5.0 mmol/kg (30 mmol/m²) and 5.5 mmol/kg (33 mmol/m²) in male and female rats, respectively. In weanling male rats, death occurred in 1 of 3 rats after administration of 10 mmol/kg. Clinical signs observed in rodents after high intravenous doses included apathy, disturbances in gait, accelerated respiration, and prostration.

In Beagle dogs, there was licking of the lips, reddening of the mucosa and skin, tremors, hematuria, disturbances in gait, retching, vomiting and bleeding at the injection site, possibly resulting from the osmotic load after administration of 6.0 mmol/kg (120 mmol/m²) at a dose volume of 12 mL/kg, but no deaths occurred. A single intravenous bolus (>10 mL/30 seconds) injection of 5 mmol/kg (60 mmol/m²) at a dose volume of 10 ml/kg was well tolerated in male and female rabbits, but a similar injection of 7.5 mmol/kg (90 mmol/m²) at a

dose volume of 15 ml/kg caused delayed death (on Day 4 after dosing) in the single rabbit administered this dose level. In another study in which single administration of 10 mmol/kg (120 mmol/m²) caused death in 4 of 4 rabbits, discoloration of the kidneys and liver was observed in some animals at necropsy. There was no clear sex difference in any animal species. When based on body weight, the minimum lethal dose levels were approximately 50 times (rodents), and 75 times (rabbits) higher than the dose of 0.1 mmol/kg recommended for clinical use in humans.

After a single intravenous administration of 0.5 mmol/kg (3.0 mmol/m2) ^{153}GdDTPA to rats, the concentration of the radiolabel was higher in the kidneys than in blood plasma. The concentration in all other tissues were lower than in plasma and decreased by ≥90% from 5 minutes to 6 hours after administration, with a tissue elimination half-life of about 17 minutes (range: 12 to 23 minutes), and the dose fraction of the radiolabel in the kidneys was 0.1% or less of total administered radioactivity by 7 days after administration. By 7 days after injection, about 90% of the radiolabel was excreted into urine and 7% was excreted into feces. There was no evidence that free gadolinium was released during passage through the body.

After administration of a higher single intravenous dose (2.5 mmol/kg;15 mmol/m2) to male Sprague-Dawley rats as Gd-^{14}C-DTPA, the radiolabel was also rapidly cleared from tissues and the concentration of Gd in tissues 24 hours after administration decreased to one tenth that measured at 10 minutes after administration. Concentrations of Gd decreased to below the limit of detection within 14 days after dosing in liver, spleen, and bone and within 60 days in the kidneys (half life approximately 5 days).

Similarly, in female Beagle dogs given a single intravenous administration of 1 mmol/kg (20 mmol/m2) ^{153}GdDTPA, the concentration of the radiolabel was higher in the kidneys than in other organs. The majority of the radiolabel was found in the renal cortex and the radioactive dose fraction in the kidneys at 7 days after administration was about 0.1% of the administered radioactivity. Within 4 hours, about 95% of the administered radiolabel was excreted into urine and about 1% was excreted into feces. By 7 days, 95.7% of the administered

radioactivity was excreted into urine and 0.67% was excreted in the feces. There was no evidence that gadolinium was released from the chelate during the passage through the organism.

In a comparative expanded single dose, **systemic tolerance** study of 2 batches of gadopentetate dimeglumine in female cynomolgus monkeys, a single intravenous injection of 0.5 mmol/kg (6.0 mmol/m²) was well tolerated, with mild, transient changes in some clinical pathology parameters in monkeys that were without histopathological correlation by Day 45. These changes included transient increases in serum iron (Day 1), slight reductions in erythrocyte (RBC) count and hemoglobin (Day 2), marked increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyltranspeptidase (GGT) in 1 of 3 monkeys that were observed on Day 2 but not on Day 43.

Single intragastric doses of up to 15 mmol/kg were well tolerated in male and female mice and male and female rats. After intrapulmonary instillation of 0.8 µmol/kg of a diluted formulation (1.0 µmol/L) to anesthetized male and female Beagle dogs, minimal to slight focal inflammatory reactions were observed at the area of application in both treated and saline-dosed dogs, but arterial blood oxygen partial pressure was not affected.

In an initial repeated dose toxicity study in male and female Wistar rats dosed at the minimum lethal dose observed in single dose studies (5.0 mmol/kg; 30 mmol/m²), gadopentetate dimeglumine was poorly tolerated when given intravenously, 5 days/week with treatment-free weekends for 4 weeks. About half of the rats died (4 of 10 males; 6 of 10 females). Clinical signs observed in rats given this dose level included reduced body weight gain, anemia [lower RBC counts, hemoglobin content and hematocrit values, and higher reticulocyte counts], higher serum ALT values, and higher liver weights, when compared with concurrent controls. Signs observed in rats given 2.5 mmol/kg (15 mmol/m²) or 5 mmol/kg (30 mmol/m²) included apathy, prone position, abnormal respiration, spasmodic convulsions, increased water intake, higher reticulocytes, and higher kidney weights, compared with controls. Gadopentetate dimeglumine was well tolerated without any organ toxic effects, after repeated administration of a lower dose level (1.0 mmol/kg; 6.0 mmol/m²).

Vacuolar change of hepatic cells was observed in rats given 5.0 mmol/kg and vacuolation of renal proximal tubular cells was observed in rats given either 2.5 or 5.0 mmol/kg (15 or 30 mmol/m²). In a follow-up study also conducted in rats given 2.5 or 5.0 mmol/kg (15 or 30 mmol/m²) 5 days/week with treatment-free weekends for 4 weeks, the vacuolar changes in the liver were reversible 8 days after the cessation of dosing. In this study, the renal vacuolation was clearly reversible in rats given 2.5 mmol/kg (15 mmol/m²) and tended to reverse in rats given 5.0 mmol/kg (30 mmol/m²), within 2 weeks after the cessation of dosing. In a third study, vacuoles in the renal proximal tubular epithelial cells and in hepatocytes were no longer present 31 weeks after 18 administrations of 5.0 mmol/kg (30 mmol/m²) given to rats over 4 weeks (5 days/week with treatment-free weekends). The vacuolations of hepatic and renal cells were interpreted as a transient storage phenomenon without long term adverse effects.

Gadopentetate dimeglumine was generally well tolerated after daily intravenous administration of 0.1, 0.5, or 2.5 mmol/kg (0.6, 3.0, and 15 mmol/m²) to male and female Jcl:SD rats daily for 5 weeks (7 days/week). During the treatment period, 1 control rat and 2 rats from the high-dose group died. After completion of treatment, no treatment-related changes were observed in the group administered 0.1 mmol/kg/day (0.6 mmol/m²/day). Dose-dependent mild to moderate vacuolation and hydropic changes of the renal tubular epithelium were observed in some animals treated with 0.5 and 2.5 mmol /kg/day (3.0 and 15 mmol/m²/day) . These changes were at least partially reversible during the 2 weeks of the recovery period. Kidney, thymus (males) and uterus weights were higher than in controls for rats given 2.5 mmol/kg/day. There were no changes in blood biochemistry parameters or the renal excretion rate, except for slightly lower values for red blood cells and hemoglobin content, compared to controls. These were reversible after cessation of treatment. Based on these findings, the no observed adverse effect level (NOAEL) after repeated intravenous injection of gadopentetate dimeglumine in rats was 2.5 mmol/kg (15 mmol/m²).

Gadopentetate dimeglumine was well tolerated after repeated (5 days/week) intravenous administration of 0.25, 1.0 or 2.5 mmol/kg (5, 20 and 50 mmol/m²) to male and female Beagle dogs for 4 weeks. No dogs died in this study. Clinical signs observed after dosing of

1.0 and 2.5 mmol/kg included transient redness of the ear and apparent nausea. After intravenous administration of 2.5 mmol/kg (50 mmol/m²), there was a slight increase in water consumption, a slight increase in reticulocyte counts in the absence of other changes in hematology parameters, slight prolongation of thromboplastin time, or slightly higher values for AST in some dogs, that were not associated with a histological alteration in the relevant tissues (liver, spleen, or bone marrow). Based on these findings, the NOAEL after repeated intravenous injection of gadopentetate dimeglumine in adult Beagle dogs was also 2.5 mmol/kg (50 mmol/m²).

Minimal or slight focal calcium deposits in the fundus mucosa or tunica muscularis of the stomach wall were sporadically observed histologically in rats that underwent long-term recovery periods [31 weeks after 18 administrations of 5 mmol/kg (30 mmol/m²) or 104 weeks after 5 or 15 daily injections of 0.5 mmol/kg (3.0 mmol/m²)]. Since the rats in which mineralization was observed had either progressive kidney lesions (progressive nephropathy) or massive neoplastic lesions, these changes were considered secondary to suspected systemic hypercalcemia and not a direct result of treatment with gadopentetate dimeglumine.

A comprehensive series of in vitro studies in bacterial systems (5 strains of S. typhimurium and E. coli WP2 uvrA) and mammalian systems (HGPRT assay in Chinese hamster V79 lung cells) suggests that gadopentetate dimeglumine is not mutagenic or clastogenic and does not induce unscheduled DNA repair in rat hepatocytes or cause cellular transformation of mouse embryo fibroblasts. Tests for **mutagenicity** were also performed in vivo with gadopentetate dimeglumine in the mouse dominant lethal assay. Treatment of male mice with dose levels up to 6 mmol/kg of body weight of gadopentetate dimeglumine failed to elicit dominant lethal mutations in offspring derived from serial mating.

Gadopentetate dimeglumine did not show any mutagenic potential in micronucleus tests after intravenous injections of 9 mmol/kg (27 mmol/m²) to mice and 2.5 mmol/kg (50 mmol/m²) to Beagle dogs.

Regarding the need for **carcinogenicity** studies, it should be noted that gadopentetate dimeglumine is a hydrophilic contrast enhancement agent intended for single use in humans that does not bind protein or penetrate plasma membranes. It distributes in the extracellular space, and is rapidly excreted from the body, without metabolism, by glomerular filtration. No carcinogenicity study with long-term administration of gadopentetate dimeglumine was conducted because of the absence of mutagenic or clastogenic potential, and the absence of regenerative or proliferative changes in tissues even after repeated administration. This is supported by the results of a study in which no neoplastic changes were noted in male and female rats that were observed for 2 years after administration of 5 or 15 daily injections of 0.5 mmol/kg (3.0 mmol/m²) gadopentetate dimeglumine. Gd content in several tissues was analyzed in this study. Gadolinium was still detectable in all investigated tissues after an observation period of 56 weeks in a range of 1.27 mmol Gd per g skin and 34.98 nmol Gd per g bone. Prolonging the observation period up to 93 weeks indicated a trend to decreased content in muscle and testes, whereas the tissues with the highest content, namely liver and kidney, showed no clear trend of a further decrease.

Intraperitoneal administration of gadopentetate dimeglumine at doses of 0.1 and 0.5 mmol/kg to male and female Sprague Dawley rats prior to mating and during early gestation did not adversely affect the health of the parental animals and had no effect on **parental reproduction** parameters. Administration of the high-dose (2.5 mmol/kg/day; 15 mmol/m²) was associated with lower than control weight gain in parental animals, and decreased testes and epididymal weights in males. No clear compound-related effects on reproduction parameters were noted. Since complete bioavailability of gadopentetate dimeglumine administered via the i.p. route was demonstrated for male rats in a comparative pharmacokinetics study, these fertility studies are considered relevant for the intravenous administration of gadopentetate dimeglumine.

Two additional studies were conducted in Sprague Dawley rats to assess the effects of gadopentetate dimeglumine on **male reproduction**. Intraperitoneal administration of gadopentetate dimeglumine (2.5 mmol/kg; 15 mmol/m²) for 60 days resulted in decreased spermatogenesis, which was not reversible during a 6-week recovery period. In the second

study, 2.5 mmol/kg gadopentetate dimeglumine was also intraperitoneally administered to male rats daily for 60 days. Male rats were serially mated to untreated females immediately and at 3, 9, or 34 weeks after cessation of dosing. Suppression of spermatogenesis (decreased number of spermatogenic cells in the testes and decreased spermatozoa in the epididymus) was noted histologically in the treated male rats starting at 3 weeks after treatment. Electron microscopy revealed atrophy of the seminiferous tubules, degeneration of the fine structure of the Sertoli cells, and swelling of the basement membrane. This suppression persisted over the entire recovery period, and was therefore considered irreversible. In two other studies, evaluation of hormone levels after 2 or 18 injections of 5.0 mmol/kg (30 mmol/m²) indicated that these changes were not hormonally mediated. No testicular changes were observed after intravenous administration of $GdCl_3$ (0.050 mmol/kg) or DTPA (0.4 mmol/kg) to male rats for 4 weeks (5 days/week with treatment-free weekends).

In a study on **female fertility**, no treatment related effects were observed after daily intravenous administration of 1.25 mmol/kg (7.5 mmol/m²) to female Sprague-Dawley rats for at least 14 days before mating, throughout mating, and until Day 7 of gestation. Some dams occasionally displayed subdued behavior immediately after dosing with the highest dose level (5 mmol/kg; 30 mmol/m²) and slight maternal toxicity (reduced body weight gain and food consumption) was observed after repeated administration of 2.5 mmol/kg or 5 mmol/kg (30 mmol/m²). There were no effects on mating performance of the females, but lower ovarian weights and slightly higher pre- and post implantation losses were observed after administration of 5.0 mmol/kg (30 mmol/m²). Exposure was confirmed by concurrent toxicokinetics analyses, for which initially high C_{max} values (ranging between 2.86 and 21.3 mmol/L across the dose levels) rapidly decreased over 4 hours after dosing. $AUC_{0\text{-}4hr}$ values increased non-linearly between the dose levels and there was no evidence of accumulation with repeated administration.

Slight retardation of ossification in fetuses, but no **maternal toxicity, embryotoxicity, or teratogenic effects** was observed in Wistar rats intravenously given 1.25 mmol/kg (7.5 mmol/m²) daily during organogenesis to assess its potential to induce embryofetal or developmental toxicity. In a second study, conducted in inseminated Jcl:SD rats using higher

dose levels (0.5 to 4.5 mmol/kg; 3.0 to 27 mmol/m²), daily intravenous injection of gadopentetate dimeglumine during organogenesis resulted in maternal toxicity, characterized by decreased food consumption (≥1.5 mmol/kg; ≥9 mmol/m²) and lower body weight gain (4.5 mmol/kg; 27 mmol/m²) compared with controls. Increased fetal mortality, decreased fetal body weight of female pups, and delayed ossification was observed after administration of the high-dose (4.5 mmol/kg; 27 mmol/m²) to the dams. No teratogenicity was induced and the postnatal development of the F1 animals and the F2 fetuses were unaffected.

Maternal toxicity, characterized by slightly decreased food consumption, was also observed in inseminated KAR:NZW rabbits intravenously administered 1.25 mmol/kg (15 mmol/m²) during organogenesis. In this study, the NOAELs for maternal and fetal effects were identified as 0.75 mmol/kg (9 mmol/m²) and >1.25 mmol/kg (>15 mmol/m²), respectively. In a second embryo-fetal toxicity study in NZW rabbits conducted at higher dose levels (0.3, 1.0 and 3.0 mmol/kg; 3.6, 12, and 36 mmol/m², respectively), no compound related effect was observed after intravenous dosing with ≤1.0 mmol/kg/day during organogenesis. Maternal toxicity (reduced body weight gain and food consumption, swelling of liver parenchyma) and decreased fetal weights and delayed ossification in fetuses but no structural malformations were observed at 3.0 mmol/kg/day (36 mmol/m²). In this study, the NOAELs for maternal and fetal effects in rabbits were identified as 1.0 mmol/kg (12 mmol/m²).

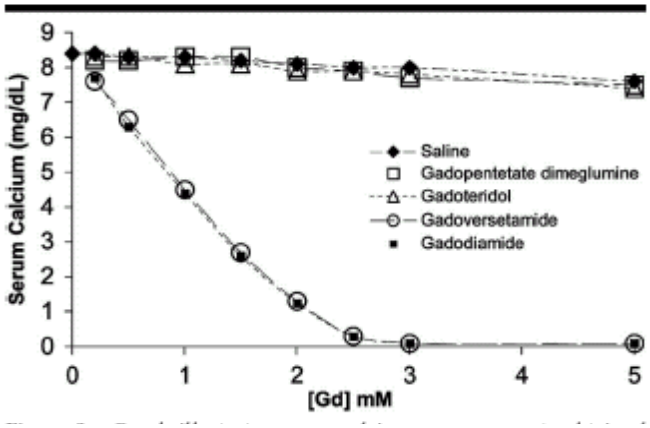
In a **peri-postnatal toxicity study**, gadopentetate dimeglumine was intravenously administered to female Jcl:SD rats at doses of 0.4, 1.2 and 3.6 mmol/kg (2.4, 7.2, and 21.6 mmol/m²) daily from Day 17 of gestation until Day 21 after delivery. In the dams given 3.6 mmol/kg (21.6 mmol/ m²) there was a transient reduction in food consumption but no net effect on the body weight or general condition was observed. In this group, suppression of body weight gain and delay in opening of eyelids were found in the offspring but no effects on the gestation period, number of implantations, number of offspring, perinatal lethality, or birthrate were observed. From these results, general toxicological NOAEL in the dams was 1.2 mmol/kg (7.2 mmol/m²), the NOAEL for reproduction was 3.6 mmol/kg (21.6 mmol/m²), and that for offspring was 1.2 mmol/kg (7.2 mmol/m²).

Gadopentetate dimeglumine induced very slight to moderate **local intolerance** in rabbits after intravenous, intra-arterial, intramuscular, paravenous, paravasal, conjunctival, or subcutaneous applications. Single injections directly into the prostate, liver, or mammary glands of rats did not reveal any compound-related findings, suggesting that leakage of diluted contrast medium to adjacent healthy tissues of these organs would be well tolerated.

No **antigenicity** was detected in the rabbit-guinea pig passive cutaneous anaphylaxis (PCA) test, a gel diffusion test using sensitized serum of rabbits (Ouchterlony's test), a mouse-rat IgE production test, and a guinea pig systemic anaphylaxis (ACA) test. A guinea pig maximization tests did not indicate that gadopentetate dimeglumine has a potential for **dermal sensitization**.

On the basis of the results of the comprehensive toxicology studies performed for the safety assessment of gadopentetate, no evidence for a risk to the health of humans at the envisaged diagnostic use for Magnevist could be deduced.

Magnevist does not interfere with serum and plasma calcium measurements determined by colorimetric assays, as illustrated in the figure below [6].



Text Figure 2: Calcium serum measurements using the OCP colorimetric assay after the addition of gadodiamide, gadoversetamide, gadoteridol, gadopentetate dimeglumine and saline

3. NSF and Gadolinium- based contrast agents – summary of background information

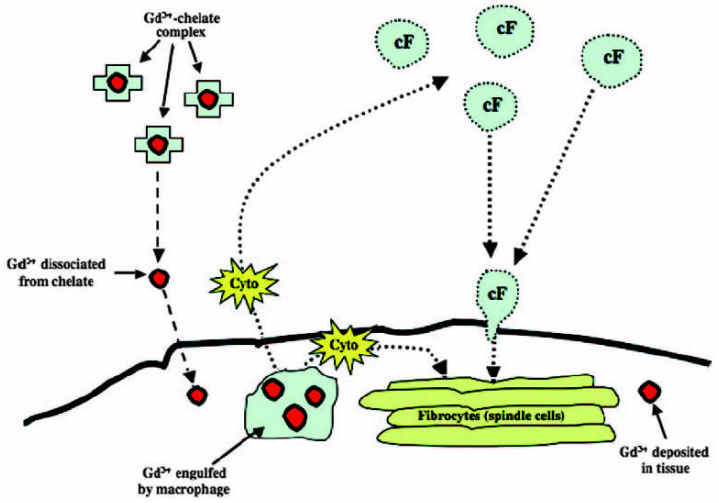
Nephrogenic systemic fibrosis (NSF), previously known as nephrogenic fibrosing dermopathy (NFD) is a systemic disease typically characterized by fibrosis of the skin and other connective tissues throughout the body (muscles and internal organs may also be affected in some patients). It was first described in the medical literature in 2000 with the first reported case dating back to 1997[7]. The majority of affected patients has CKD stage 5 (usually dialysis dependent renal failure), a smaller proportion CKD stage 4 or acute renal failure. There are very few reports of NSF in patients reportedly having CKD stage 3. Symptoms of NSF may include thickening of the skin, swelling of the lower extremities, redness, pruritus, and burning sensations. In approximately 5% of patients, the course of the disease is rapidly progressive and may potentially lead to a fatal outcome[8]. Definitive diagnosis of NSF requires deep skin biopsy and histopathology.

Based on current information, it appears that males and females are affected in approximately equal numbers. The onset of NSF is generally during middle age, although pediatric cases

have also been reported. Currently, there is no known cure for NSF. Improving renal function seems to slow or arrest NSF and may even result in a gradual reversal.

The etiology of NSF is still unknown but is thought to be multifactorial. The particular combination and severity of co-factors necessary to trigger the development of NSF has not, as yet, been elucidated. Specific triggers under scientific evaluation have included surgery and/or the occurrence of thrombosis or other vascular injury[9], proinflammatory state[10], the administration of high doses of erythropoietin[11] , and the use of GBCAs [12 13 14 15 16]. However, reports of NSF in patients without any known GBCA-exposure have also been published[17 18 19].

The prevailing theory regarding gadolinium and NSF is that gadolinium (Gd^{3+}) ions are released from the Gd-chelate complex of MRI contrast agents and accumulate in tissues such as skin, thereby initiating what some have described as a “toxic” reaction. The precise pathomechanism is not yet known (Text Figure 3).[20]



Text Figure 3: Speculative mechanism by which gadolinium (Gd^{3+}) might trigger nephrogenic systemic fibrosis.

In the setting of kidney disease, impaired renal excretion of (Gd^{3+}) prolongs the half-life and enhances the chance for dissociation of (Gd^{3+}) from its chelate, allowing increased tissue exposure. Vascular trauma and endothelial dysfunction allow free (Gd^{3+}) to enter tissues more easily, where macrophages phagocytose the metal and produce local profibrotic cytokines as well as signals that attract circulating fibrocytes to the tissues. Once in tissues, circulating fibrocytes induce a fibrosing process that is indistinguishable from normal scar formation.[20]

The likelihood of a particular Gd-chelate to release Gd^{3+} ions is thought to depend at least in part on the chelate's physicochemical properties. Some of these Gd^{3+} ions are thought to accumulate in the body in the case of reduced renal elimination.

In order to evaluate potential differences between various GBCAs regarding their likelihood to trigger NSF-like symptoms in patients with severe renal impairment, the available clinical evidence for Gd-based contrast agents, including number of reports, published studies, range of approved indications, range of dosages approved for use in CE-MRI, and number of administrations and years since initial approval should be considered. In addition, based on the prevailing theory on the possible role of GBCAs in the development of NSF, the following factors should also be considered:

- Complex stability of Gd-based contrast agents
- Pharmacokinetics of Gd-based contrast agents
- Results of non-clinical exploratory studies intended to evaluate possible differences between Gd-based contrast agents regarding their potential risk to trigger NSF-like skin changes.

These data will be described in more detail in the following sections.

4. Clinical evidence

4.1 Introduction

A possible link between gadolinium-based contrast agent administration and development of nephrogenic systemic fibrosis was first suggested by Grobner in 2006 [21]. The first published report on NSF appeared in 2000[7]. Researchers had identified cases dating back to 1997. Until the publication by Grobner, however, no one had suggested that gadolinium contrast administration may have a possible role in the development of NSF.

In July 2006, the company recorded its first case report of NSF in a patient who had possibly received Magnevist. Since that time, through the data lock point for this review (30 Sep 2009), the company has received 554 reports of NSF or NSF-like symptoms in patients who received or who reportedly received Magnevist. The majority of these reports are lawsuits filed against Bayer and often against some or all of the other manufacturers of gadolinium-based contrast agents. Many of the reports contain only minimal information.

The Text Table 4 shows number of cases received each quarterly period since the first case was received in July, 2006.

Text Table 4: Number of cases received by quarter

Quarterly Period	Number of Cases Received
July – August – September 2006	5
Oct – Nov – Dec 2006	8
Jan – Feb – Mar 2007	41
Apr – May – June 2007	31
July – August – September 2007	11
Oct – Nov – Dec 2007	15
Jan – Feb – Mar – 2008	36
Apr – May – June 2008	62
July – August – September 2008	84
Oct – Nov – Dec -2008	63
Jan – Feb – March 2009	58
Apr – May – June 2009	92
July – August – September 2009	48
TOTAL	**554**

Of note, however, the time period during which the case is reported is usually not equal to the onset of the disease. While case reports continue to be received, they are primarily describing cases with an earlier onset (Text Table 7).

Bayer applies the following General Principles to its handling of NSF case reports:

1. All events that are reported to the company as nephrogenic systemic fibrosis (NSF) or nephrogenic fibrosing dermopathy (NFD), or as possible or suspected NSF or NFD, are entered, coded, and reported to global health authorities as NSF, regardless of the source of the report or the degree of documentation it contains. No "confirmation" of an NSF diagnosis is required to warrant a case's inclusion in the global safety database. As with other spontaneously reported events, coding and reporting of NSF cases is based on the verbatim reported terms and not on the company's opinion or internal characterization of these reports.

2. As there are many cutaneous disorders which occur in patients with renal impairment, some of which have clinical and histological features similar to NSF, in order to more soundly evaluate the cases reported as NSF associated with their products, Bayer apply the guidelines for a standardized NSF definition established by a working group of experts (unpublished communication Cowper). These experts developed a scoring system based on both a clinical and a histopathological assessment. With this in mind, some cases that have been reported to the company as "NSF" bear little resemblance to the characteristics described as necessary components of this diagnosis as outlined in the standardized definition. While such cases are nevertheless entered in the database, coded, and reported to regulatory authorities as NSF, it is strongly believed that not all cases truly represent NSF.

3. After publication of the Grobner article, Bayer regularly searched the FDA's AERS database for cases of NSF/NFD that had been reported in association with the use of any pharmaceutical product. Company pharmacovigilance practices then required that any case reported in association with a Bayer product, must be entered into the Bayer global safety database if the report could not be matched with an existing case. Thus, the Bayer global safety database contains reports derived from the FDA's AERS database line listings for which the available information is so minimal that it precludes a proper duplicate search. Bayer therefore believes that their database may contain duplicate NSF reports.

4. Bayer enters cases derived from lawsuits even though in a significant number of these, it appears that it is not yet known which product(s), if any, were actually administered or whether a diagnosis of NSF has been confirmed. However, the language in the legal complaints is broad and implies allegations against all named defendants (for example, "After the defendants' products were administered . . . or "After receiving Magnevist AND/or product X, . . ."). While it could be argued that such legal complaints do not meet one of the four minimal criteria for a case, for instance a known suspect drug, Bayer has taken a conservative approach and has filed these reports pursuant to FDA regulations (eg, 21 CFR 314.80), whereby the filing of such AE reports does not necessarily reflect a conclusion that the particular gadolinium-based contrast agent caused or contributed to the reported adverse event.

Bayer understands that adherence to the above principles has likely resulted in an overestimated number of case reports of NSF associated with Magnevist. Since other GBCA manufacturers may take a different approach, caution should be exercised when directly comparing raw numbers of case reports associated with individual GBCAs.

4.2 Summary of reports of NSF in association with the administration of Magnevist®

Of the 554 case reports received and evaluated by Bayer Global Pharmacovigilance through the data lock point for this review, 233 of these cases contained no documented administration of Magnevist and will be excluded from further analysis pending receipt of additional information. The majority of these are legal complaints in which it is alleged, for example, that on unspecified dates Magnevist and/or other products "might have" been administered, after which the plaintiff (also often on an unknown date) developed NSF.

In the remaining 321 cases, patients were reported to have received Magnevist alone or in combination with other products; 142 of these reports were confounded by the administration of other GBCAs in the same timeframe in which NSF could plausibly have developed.

4.2.1 Assessment of Patient Population – Cumulative Reports

The 321 reports of possible NSF or NSF-like symptoms received by the Global Pharmacovigilance department, in which Magnevist administration was reported, originated from 15 countries, as seen in Text Table 5, with a disproportionate 85% of all reports originating from the United States.

Text Table 5: Country origin of NSF reports in association with administration of Magnevist

Reports of NSF/NSF-like symptoms received through 30 Sep 2009: Breakdown by Country of Origin in Reports with Documented Magnevist Administration	
Country	**Number of Cases**
Austria	3
Belgium	1
Bermuda	1
Canada	2
Denmark	8
France	2
Germany	15
Great Britain	2
Italy	1
Japan	6
The Netherlands	2
Norway	2
Spain	1
Switzerland	1
United States	274
Total	**321**

Evaluation of the 321 case reports indicates that 159 of the 321 patients (49.5%) were male, 131 (40.8%) were female, and in 31 cases (9.7%), gender was not specified. Race was identified in 104 of the 321 reports as follows: 72 patients were Caucasian; 24 patients were African-American or Black; two patients were Asian; two were Hispanic; and two were "Other". Race was not specified in the remainder of the cases. Ages, where provided, ranged from 11 years to 92 years; with females ranging from 26 - 85 years (median age 56 years) and males ranging in age from 11 to 92 years (median age 58 years). The ages of 51 males and 35 females were not provided.

Medical Confirmation (Healthcare professional reports)

Of the 321 reports, 220 were from healthcare professionals.

Gadolinium Exposure

In the 321 cases received through the data lock point for this review, in which Magnevist administration was identified, 77 patients received at least one GBCA administration while one patient received as many as 38 gadolinium-enhanced procedures. Where the specific number of gadolinium-enhanced procedures cannot be surmised, it is counted as "at least one."

The 321 patients received a total of at least1,265 gadolinium-enhanced procedures, for an average of 3.94 procedures per person. At least 945 of these involved Magnevist, with each patient receiving from at least one to, in one case, as many as 33 doses of Magnevist.

Dosing

While dosing information is often not provided, or is at best incomplete, many patients received doses that far exceeded the approved dose of 0.1 mmol/kg. Where weight-based dosing could be calculated, doses ranged from the approved 0.1 mmol/kg to 0.6 mmol/kg. Individual dose volumes ranged to 110 mL, with cumulative gadolinium exposure in an individual ranging to 380 mL. Due to missing dose information no cumulative dose could be calculated in a large number of patients.

Product Identification

In 179 of the 321 reports (55.8%), Magnevist was the only product reported (Text Table 6). The other reports were confounded by administration of other known and unknown GBCAs, including Dotarem*, Gadovist*, Multihance, Optimark, Omniscan, Prohance, and "unspecified GBCAs" in various permutations and combinations.

* not marketed in the US

Text Table 6: Product identification in NSF reports received by Bayer

PRODUCTS ADMINISTERED								NUMBER
MV	OS	OM	MH	PH	GV*	DT*	GBCA NOS	
X								179
X	X							57
X	X	X						3
X	X	X					X	2
X	X		X					2
X	X			X				4
X	X					X		3
X	X						X	16
X	X		X				X	1
X	X			X		X		1
X	X	X	X					1
X		X						4
X		X					X	3
X			X					4
X			X				X	3
X				X				2
X					X			2
X						X		1
X							X	32
X					X	X	X	1

MV = Magnevist; OS = Omniscan; OM = Optimark; MH = Multihance; PH = Prohance; GV = Gadovist; DT = Dotarem; GBCA NOS = as yet unidentified GBCA
* not marketed in the US

Text Table 6 illustrates that in the 321 cases in which Magnevist was administered, Omniscan was also used in 90 patients; Optimark was used in 13 patients; Multihance was used in 11 patients; Prohance was used in seven patients; Gadovist (gadobutrol) was used in 3 patients, Dotarem (gadoterate) was used in 6 patients, and an as yet unidentified GBCA was administered to 58 patients. In 96 cases, the product administered in closest temporal association to onset of NSF-like symptoms was something other than Magnevist.

Time to Onset of symptoms in relation to gadolinium exposure

Time to onset of signs and symptoms suggestive of NSF in relation to gadolinium exposure ranged from the "same day" or "within days" of exposure to years later (in one case, 11.5 years), and in 74 cases was unknown or not reported. In 4 cases, symptoms seemed to predate gadolinium administration. In 166 patients, onset of symptoms occurred within one year of a gadolinium exposure. In 76 of these patients, symptom onset occurred within one month of exposure.

Disease Onset

Although Bayer has received some isolated and sometimes anecdotal reports of NSF symptoms starting in a patient as early as 1998 (relying for example on patients' memories), first documented onsets date back to about 2000. At that point in time (beginning of 2000), Magnevist had been on the market for 12 years in dozens of countries all around the world, and had been administered during approximately 31 million procedures (more than most of the currently marketed GBCAs to date). As the first GBCA, Magnevist was the most used and studied MR contrast agent in the world, and the subject of countless scientific publications.

Text Table 7 illustrates the year of onset of NSF-like symptoms for the 321 cases that currently document a Magnevist administration. If the year of symptom onset is completely unknown, the year of diagnosis or biopsy is used. Although the cases have only been received beginning in 2006, diagnosis of the disease dates back to before the time that anyone had suggested a connection between it and gadolinium administration.

Text Table 7: Year of onset of NSF-like symptoms after administration of Magnevist

Onset of signs and symptoms suggestive of NSF by year in all cases in which Magnevist was administered through Sep 30, 2009	
Year of onset of signs and symptoms	**Number of Reports**
1998	1
1999	1
2000	4
2001	0
2002	15
2003	3
2004	40
2005	44
2006	61
2007	59
2008	23
2009	2
Unknown or unclear	68
TOTAL	**321**

Deaths

Of the reports of NSF/NSF-like symptoms reported to Bayer Global Pharmacovigilance through Sep 30, 2009, in which Magnevist was reported to have been administered, 71 patients were reported to have died.

4.2.2 Assessment of Possible Association to Magnevist

In terms of standardized assessments, and to assist with individual case evaluations for spontaneous reports, the Bayer Global Pharmacovigilance database in use since late November 2007 (Clintrace) contains a built-in algorithm, which takes into account the temporal association to the product, dechallenge and rechallenge information (generally not

applicable to contrast media administration), the reporter's assessment, and possible alternative explanations for the reported event(s). This results in algorithm assessments of "not excluded", "excluded", and "not assessable." In the database previously used by the company (Argus), these terms translated to "possible", "unlikely" and "unclassifiable". "Not excluded" is a very broad term, and includes most cases for which the drug was reported to have been given, and the event occurred afterwards. "Not assessable" is generally reserved for cases in which there is very little information (eg, the product/products administered are not known, administration dates are not known, and/or symptom onset or diagnosis date is not known).

For clinical trials, post marketing studies, and solicited reports, assessment categories in the Clintrace database are limited to "associated" and "not associated." Cases that are "associated" are equivalent to those that are "not excluded" or "possibly related." For cases that are "not associated", a relationship is "excluded" or "unlikely."

To assess the strength of the possible association, the following criteria are also taken into account, based on Broome et al.[15]

1. The diagnosis of NSF should be confirmed by skin biopsy and should not be based solely on clinical manifestations.

2. The specific MR contrast agent should be documented in the contrast administration records and should not simply be inferred.

3. The temporal relationship of contrast administration and development of NSF should be documented and should likely conform to the time period of 2 weeks to 3 months reported in this and other series. *Bayer HealthCare Pharmaceuticals has taken the significantly longer period of 18 months for consideration of a temporal association.*

4. The possibility that patients may have been exposed to multiple MR contrast agents during the 3-month period should be verified, including those administered at other facilities.

As of Sep 30, 2009, Bayer Global Pharmacovigilance and its global affiliates had received and evaluated 554 reports of patients who had reportedly developed NSF or NSF-like symptoms following reported GBCA administration. For the majority (358/554, or 64.6%) of these reports, the currently available information is insufficient to establish a temporal association to Magnevist injection and/or confirm the diagnosis of NSF via skin biopsy or other means and/or confirm that Magnevist was specifically administered. For these reports, association with Magnevist is considered excluded or not assessable unless and until additional information is provided. Two recent reports of NSF occurring in patients with mild to moderate renal impairment are considered unconfirmed due to lack of information.

For the remaining 196 reports, based on the information currently available, and taking into account the Broome criteria, in 98 of these reports, association to Magnevist was considered possible, based primarily on a temporal association (18 months or less) between documented and generally unconfounded Magnevist administration and onset of symptoms, lack of a plausible alternative explanation, and confirmation of a diagnosis of NSF via skin biopsy or other means (eg, a clinical diagnosis based on patient history and symptom presentation). In an additional 98 reports, although association with Magnevist could not be absolutely excluded (primarily because the product was administered in temporal association to event onset), other etiologies were considered possible (eg, some cases were confounded by the administration of other known and unknown GBCAs in a similarly plausible temporal relationship; in some cases there was no apparent temporal relationship to Magnevist injection; and in other cases the diagnosis was not biopsy confirmed and/or included the possibility of other differential diagnoses). Included among the cases where association with Magnevist was considered "not excluded" are two cases of NSF occurring in patients who did not have severe renal impairment at the time of gadolinium administration. In one case, although there is a temporal association to the onset of symptoms, biopsy results were non-specific, and differential diagnoses included eczematous dermatitis. The other case, derived from the Japanese literature, is confounded by the administration of Omniscan and Prohance in addition to Magnevist, with Prohance being the most temporally associated to symptom onset.

4.2.3 Characteristics of Possibly Associated Reports

In the 98 case reports of NSF/NSF-like symptoms currently assessed as "possibly associated" with the reported administration of Magnevist, 61 patients (62.2%) were male, 34 (34.7%) were female, and in 3 patients (3.1%), gender was not specified. Patients' ages, where provided (n = 76), ranged from 15 years to 82 years (median age = 59.5 years) overall. For females, the age range was 30 years to 80 years in the 28 patients where age was reported (median age 59.5 years) and for males the age range was 15 years to 82 years in the 47 patients where age was provided (median age 60 years). In two patients, neither age nor gender was provided.

Medical Confirmation (Healthcare professional reports)

Of the 98 possibly associated reports, 71 (72.4%) were from healthcare professionals.

Renal and Dialysis Status

Seventy of these patients (71.4%) were on dialysis at the time of GBCA administration/ onset of NSF-like symptoms (50 on hemodialysis, 10 on various forms of peritoneal dialysis, and 10 alternately receiving both HD and PD; with length of time on dialysis varying greatly, from less than one year to more than 20 years). One patient had stage 4 chronic kidney disease with GFR < 30 mL/minute, but did not start dialysis until after Magnevist administration. One patient was failing a kidney transplant but did not restart HD until after NSF onset. Other patients had varying descriptions and degrees of renal impairment with no reference to dialysis.

Etiology of Renal Impairment

The most frequently noted causes of renal impairment in these patients were: diabetes mellitus, glomerulonephritis, hypertension, IgA nephropathy, and congenital kidney disorders or pediatric kidney disease. Other etiologies included multiple myeloma, infections and sepsis, focal segmental glomerulosclerosis, lupus nephritis, and post partum hemolytic uremic syndrome. One patient had renal failure in the context of the hepatorenal syndrome.

In many patients the cause of renal insufficiency was noted to be multifactorial; in the majority of patients, it was unknown or not specified.

Comorbidities

Many cases, even those considered possibly associated, still contain very minimal information. However, where medical history and concomitant medical conditions were provided, all patients were multimorbid. Cardiac, cardiovascular, and endocrine diseases were common. Progression of kidney disease led to disorders of calcium and phosphorus metabolism, hyperparathyroidism, hyperphosphatemia, metabolic abnormalities, anemia, and bone disease. At least 28 patients had undergone kidney transplantation, some as many as three times; two patients had undergone liver transplantation. At least seven patients had experienced reactions, some severe, to iodinated contrast media, which provided some potential insight into why some of these patients received gadolinium-based contrast for x-ray procedures and during interventional radiology. Thirty-one patients also had multiple other documented allergies, some severe. Twenty-seven of the patients had various types of cancer. Dialysis patients often received erythropoietin, phosphate binders, and intravenous iron supplementation. Many patients' clinical courses were complicated by multiple episodes of infection, recurrent clotting of the dialysis access, and graft and fistula revision.

Gadolinium-enhanced procedures

Patients in the 98 possibly associated reports received from at least one to "more than" 15 Gd-enhanced procedures as indicated in the Text Table 8.

Text Table 8: Number of Gd-enhanced procedures in NSF reports associated with administration of Magnevist

Number of Gd-enhanced Procedures	Number of Patients
1 or “at least 1”	21
2 or “at least 2”	23
3	13
4	11
5	13
6	6
7	4
8	2
9	1
10	1
11	1
12	0
13	1
14	0
“more than” 15	1
Total	**98**

The 98 patients received at least 353 gadolinium-enhanced procedures (average 3.6 procedures per patient). Most of these involved Magnevist. Dosing information was often unfortunately not available (even in some cases with medical records) or was incomplete; however, where provided, patients received Magnevist at individual dose volumes ranging from 15 mL to 80 mL, and from a standard 0.09 mmol/kg dose in one Japanese case to 0.6 mmol/kg. Only 3 patients were reported to have received a "single dose" of Magnevist prior to onset of NSF symptoms.

Biopsies

The diagnosis of NSF was reportedly confirmed by skin biopsy in 76 of these 98 cases; in 14 of the biopsy-proven cases, however, neither the report nor details of the report were provided. NSF was "diagnosed" by other or unspecified means in the rest. In several cases a clinical diagnosis of NSF was made on the basis of a non-specific biopsy report.

Time to Onset of Symptoms from Magnevist Administration

In the 98 reports considered possibly associated with Magnevist administration, onset of NSF symptoms occurred within days to within approximately 18 months from the last dose of Magnevist administration (Text Table 9). Seven patients reported an onset within one week of the last administration; 34 within one month of the last administration; and 10 within three months of administration (ie, 51/98 experienced onset of NSF-like symptoms within three months of the last GBCA administration). Of note, however, many of these patients had received Magnevist without incident over a period of years prior to the onset of NSF-like symptoms.

Text Table 9: Year of Onset of NSF-like Symptoms.

Onset of signs and symptoms suggestive of NSF by year in cases considered possibly associated with Magnevist administration	
Year of onset of signs and symptoms	**Number of Reports**
1998	1
1999	0
2000	1
2001	0
2002	7
2003	6
2004	14
2005	12
2006	25
2007	26
2008	3
2009	0
Unknown or unclear	3
TOTAL	**98**

4.2.4 Single Case Reports of Magnevist and NSF in the Published Literature

To date, approximately 90 cases of NSF in patients who received Magnevist have been documented in the medical literature. These cases are contained in the articles listed in Text Table 10:

Text Table 10: Single Case Reports of Magnevist and NSF in published literature (I)

Author	Title	Journal	GBCA(s)	No. of NSF cases
Abujudeh H.H., Kaewlai R., Kagan A., Chibnik L.B., Nazarian R.M., High W.A., Kay J.[22]	Nephrogenic systemic fibrosis after gadopentetate dimeglumine exposure: Case series of 36 patients	Radiology 253, 1: 81-89 (**2009**)	Magnevist	**36** (Magnevist)
Bainotti S., Rota E., Bertero M., Tamburrini O., Balducci A., Formica M.[23]	Nephrogenic systemic fibrosis: the first Italian gadolinium-proven case	Clinical Nephrology, **2008**, 70: 514-517	Magnevist	**1** (Magnevist)
Caravan P., Koreishi A., Kay J.[24]	Post-mortem ICP-MS and MR analysis of gadolinium concentration and distribution in three confirmed NSF cases	Proc Intl. Soc. Mag. Reson. Med., **2009** (17): 402	Magnevist	**3** (Magnevist)
Deo A., Fogel M., Cowper S.[25]	Nephrogenic Systemic Fibrosis: A Population Study Examining the Relationship of Disease Development to Gadolinium Exposure	Clin J Am Soc Nephrol **2007,** 2: 264-267,	Magnevist Omniscan	**3** (2 Omniscan, 1 Magnevist)
Grebe S.O., Borrmann M., Altenburg A., Wesselman U., Hein D., Haage P.[26]	Chronic inflammation and accelerated atherosclerosis as important cofactors in nephrogenic systemic fibrosis following intravenous gadolinium exposure	Clin Exp Nephrol, **2008**, 12: 403-406	Magnevist Omniscan	**1** (Magnevist/ Omniscan)
Heinz-Peer G., Neruda A., Watschinger B., Vychytil A., Geusau A., Haumer M., Weber M.[27]	Prevalence of NSF following intravenous gadolinium-contrast media administration in dialysis patients with endstage renal disease	European Journal of Radiology: (**2009**) (ahead of print)	Magnevist Omniscan Dotarem	**3** (2 Magnevist/ Omniscan; 1 Magnevist/ Omniscan/ Dotarem)
Hope T.A., Herfken R.J., Denianke K.S., LeBoit P. E., Hung Y.-Y., Weil E.[28]	Nephrogenic Systemic Fibrosis in Patients With Chronic Kidney Disease Who Received Gadopentetate Dimeglumine	Investigative Radiology, **2009**, 44:	Magnevist	**1** (1 Magnevist + 3 unconfirmed cases)

continued

Text Table 11: Published Literature (continued)

Author	Title	Journal	GBCA(s)	No. of NSF cases
Imai C., Kurihara K., Ito M.[29]	A case of nephrogenic fibrosing dermopathy	Clinical dermatology, **2008** (50):1235-1238	Magnevist Omniscan	**1** (Magnevist/ Omniscan)
Kay J., Bazari H.; Avery L.L., Koreishi A.F.[30]	Case 6-2008: A 46-Year-Old Woman with Renal Failure and Stiffness of the Joints and Skin	N Engl J Med **2008**, 358: 827-838	Magnevist	**1** (Magnevist)
Kreuter A., Gambichler T., Weiner S.M., Schieren G.[31]	Limited Effects of UV-A1 Phototherapy in 3 Patients with Nephrogenic Systemic Fibrosis	Arch Dermatol, **2008** (144): 1527-1529	Omniscan Magnevist Dotarem	**3** (1 Omniscan, 1 Magnevist, 1 Omniscan/ Magnevist/Dotarem)
Miyamoto J., Kasai H., Takae Y.[32]	A case of a patient with nephrogenic systemic fibrosis	Japanese Dermatological Association Journal, **2009** (119): 751	Magnevist Omniscan	**1** (Magnevist/ Omniscan)
Nakai K., Takeda K., Kimura H.[33]	Nephrogenic systemic fibrosis in a patient on long-term hemodialysis	Clin Nephrol, **2009** (71): 217-220	Omniscan Magnevist	**1** (Omniscan/ Magnevist)
Othersen J.B., Maize J.C., Woolson R.F., Budisavljevic M.N.[34]	Nephrogenic systemic fibrosis after exposure to gadolinium in patients with renal failure	Nephrol. Dial. Transplant **2007**, 22:3179-3185 (Erratum 2008)	Magnevist Omniscan	**5** (4 Omniscan, 1 Magnevist)
Perez-Rodriguez J., Lai S., Ehst B.D., Fine D.M., Bluemke D.A.[35]	Nephrogenic Systemic Fibrosis: Incidence, Associations, and Effect of Risk Factor Assessment-Report of 33 cases	Radiology, **2009**, 250: 371-377	Magnevist, Omniscan	**33** (20 Omniscan, 7 Magnevist, 6 unknown)
Pieringer H., Schumacher S., Schmekal B., [36]	Gadolinium-based contrast agents, erythropoietin and nephrogenic systemic fibrosis in patients with end-stage renal failure	NDT Plus, **2008** (3): 193	Magnevist Omniscan	**4 (**Magnevist/ Omniscan)

Text Table 11: Published Literature (continued)				
Author	**Title**	**Journal**	**GBCA(s)**	**No. of NSF cases**
Schieren G., Tokmak F., Lefringhausen L. , Van Bracht M., Perings C., Willers R., Günsel A., Kemper F., Wiesmüller G.A., Rump L.C.[37]	C-Reactive Protein Levels and Clinical Symptoms Following Gadolinium Administration in Hemodialysis Patients	Am J Kidney Dis, **2008**, 51: 976-986	Magnevist	**1** (Magnevist/ Multihance)
				continued
Schietinger B.J., Brammer G.M., Wang H., Kramer CH. M.[38]	Patterns of Late Gadolinium Enhancement in Chronic Hemodialysis Patients	JACC: Cardiovascular Imaging, **2008** (4): 450-456	Magnevist Omniscan	**1** (1 Magnevist/ unknown)
Schroeder J.A., Weingart C., Coras B., hausser I., Reinhold S., Mack M., Seybold V., Vogt T., Banas B., Hofstaedter F., Kraemer B.K.[39]	Ultrastructural Evidence of Dermal Gadolinium Deposits in a Patient with Nephrogenic Systemic Fibrosis and End-Stage Renal Disease	Clinical Journal of the American Society of Nephrology, **2008** (3): 968-975	Magnevist	**1** (Magnevist)
Shabana W.M., Cohan R.H., Ellis J.H., Hussain H.K., Francis I.R., Su L.D., Mukherji S. K., Swartz R.D.[40]	Nephrogenic Systemic Fibrosis: A Report of 29 cases	AJR **2008**, 190:763-741	Magnevist (>90% of applications) Multihance Omniscan	**29** (2 Omniscan, 1 Magnevist, 22 unknown GBCA, 4 no GBCA)
Shibuya[41]	Nephrogenic systemic fibrosis (NSF)	Japanese Journal of Clinical Dialysis 25 (7): 159-166 (**2009)**	Magnevist	**1** (Magnevist)
Shin K., Granter S.R., Coblyn J.S., Gupta S.[42]	Progressive arm and leg stiffness in a patient with chronic renal impairment	Nat Clin Pract Rheumatol, **2008** (10): 557-562	Magnevist Multihance	**1** (Magnevist/ Multihance)
Su H.S., Nazarian R.M., Scott J. A.[43]	Appearance of Nephrogenic Fibrosing Dermopathy on a Bone	BJR, **2009** (82): e35-e36	Magnevist	**1** (Magnevist)

Text Table 11: Published Literature (continued)				
Author	**Title**	**Journal**	**GBCA(s)**	**No. of NSF cases**
	Scan			
				continued
Thakral C., Alhariri J., Abraham J.L.[44]	Long-term retention of gadolinium in tissues from nephrogenic systemic fibrosis patient after multiple gadolinium-enhanced MRI scans: case report and implications	Contrast Media & Molecular Imaging, **2007**, 2: 199-205	Magnevist	**1** (Magnevist)
Todd D.J., Kagan A., Chibnik L.B., Kay J.[18]	Cutaneous Changes of Nephrogenic Systemic Fibrosis-Predictor of Early Mortality and Association With Gadolinium Exposure	Arthritis & Rheumatism, **2007**, 56:3433-3441	Magnevist	**25** (16 Magnevist)
Van der Meij N., Keur I., Van Lienden K.P., Scheepstra C.G., Bos J.D.[45]	Nefrogene systemische fibrose, mogelijk veroorzaakt door gadoliniumhoudend contrastmiddel	Ned Tijdschr Geneeskd, **2007** (151): 2898-2903	Magnevist Omniscan	**1** (1 Magnevist/ Omniscan)
Weigle J.P., Broome D.R.[46]	Nephrogenic systemic fibrosis: chronic imaging findings and review of the medical literature	Skeletal Radiol, **2008**, 37: 457-464	Magnevist Omniscan	**3** (2 Omniscan, 1Magnevist)
Wertman R., Altun E., Martin D.R., Mitchell D.G., Leyendecker J.R., O'Malley R. B., Parsons D.J., Fuller E.R., Semelka R.C.[47]	Risk of Nephrogenic Systemic Fibrosis: Evaluation of Gadolinium Chelate Contrast Agents at Four American Universities	Radiology, **2008**, 248:799-806	Magnevist Omniscan	**36** (32 cases in centers where Omniscan was used, 4 cases in centers where Magnevist was used)
Yanagida T., Kawamura Y., Hase S., et al.[48]	A case of nephrogenic systemic fibrosis caused by repeated contrast-enhanced MRI in a patient with stage 3 chronic kidney disease (CKD) despite renal function assessed by estimated glomerular filtration rate (eGFR) more	Proceedings of the 39th (the year **2009**) East-Japan Regional Annual Scientific Meeting of the Japanese Society of Nephrology	Magnevist Omniscan Prohance	**1** (Magnevist, Omniscan, Prohance)

Text Table 11: Published Literature (continued)				
Author	**Title**	**Journal**	**GBCA(s)**	**No. of NSF cases**
	than 30 mL/min/1.73 m^2	(2009.10.2-3)/The Japanese Journal of Nephrology 51(6)676/(2009.8)		

In addition to the case reports described above, 4 articles on patients who developed NSF in association with GBCAs were published with no identification of the contrast agent(s) used (Text Table 11). In the articles cited Magnevist was later reported to have been admininistered.

Text Table 11: Single Case Reports of Magnevist and NSF in published literature (II)

Author	Title	Journal	No of NSF cases
Krous H., Breisch E., Chadwick A.[49]	NSF with multiorgan involvement in a teenage male after lymphoma, Ewing's sarcoma, end stage renal disease and hemodialysis	Pediatric and Developmental pathology, **2007** (10): 395-402	1 (multiple GBCAs)
Moschella S. L., Kay J., Mackool B., Liu V.[50]	Case 35-2004:A 68-Year-Old Man with End-Stage Renal Disease and Thickening of the Skin	N Engl J Med, **2004** (351): 2219-2227	1 (Magnevist)
Farlow J.T.[51]	The Enigma of Nephrogenic Systemic Fibrosis	Nephrology Nursing Journal, **2007** (34)	1 (Magnevist)
Gulati A., Harwood C.A., Raftery M., Cerio R., Ashman N., Proby C.A.[52]	Magnetic resonance imaging with gadolinium enhancement in renal failure: a need for caution	International Journal of Dermatology **2008**, 47: 947-949	1 (Magnevist/ unknown GBCAs)

Furthermore, the following publication was published as a slide presentation in which one patient with NSF in association with Magnevist was reported:

Author	Title	Journal	GBCA	No of NSF cases
Artunc F., Schanz S., Metze D., Heyne N.[53]	Nephrogene systemische Fibrose	Deutsche Medizinische Wochenzeitschrift, **2008**, 133 (F1)	Magnevist	**1** (1 Magnevist)

4.3 Summary of data on incidence rates of NSF in relation to clinical use of GBCAs

4.3.1 Introduction

In an effort to obtain more reliable data to assess the potential risk of development of NSF in relation to the use of the individual GBCAs, several studies have been conducted and published that report on the incidence of NSF following the administration of different contrast agents at various institutions. Some variability in results from institution to institution is due to the fact that different criteria were used to diagnose patients with NSF. The results of some studies demonstrated that different GBCAs are associated with varying benchmark incidences of NSF[47 18 54 28].

Furthermore, the FDA has requested from all GBCA manufacturers a post-marketing commitment to conduct a phase IV study to assess the magnitude of the potential risk for the development of NSF with each of the marketed GBCAs in patients with moderate to severe renal impairment. Bayer has initiated such studies for each of its marketed GBCAs (ie, Magnevist, Eovist and Gadovist (the latter not marketed in the US)). A status update on these ongoing studies will be provided in the following sections.

4.3.2 Summary of data on Magnevist

4.3.2.1 Published study data

The medical literature reflects a range of possible incidence rates of NSF following the administration of GBCAs. This wide range can be attributed to a variety of reasons, including: (i) study design differences, (ii) number of patients included in the analysis, with

some studies evaluating all patients who received GBCA in a certain time window, including those with normal kidney function, while others limited the analysis to patients with impaired renal function or to patients on dialysis at the time of contrast media application; and (iii) criteria used to diagnose NSF. As to the criteria used, there is currently no globally accepted definition of NSF in the medical literature, with some authors diagnosing NSF based on clinical findings alone, while other used a combination of clinical and histopathological findings.

Incidence of NSF in possible association to Magnevist administration was described in most studies to be related to the dose administered, the cumulative amount of GBCA (Magnevist, as well as other agents) administered, the renal status of the patient and potential other co-factors.

Text Table 12 provides a summary of the available study literature with Magnevist:

Text Table 12: Summary of published study results on clinical use of Magnevist and occurrence of NSF

Publication	GBCA described in association with NSF	NSF cases reported	Estimated NSF incidence	Criteria for NSF diagnosis	Remarks
Deo et al (Clin J Am Soc Nephrol **2007**)[25]	Gadodiamide, Gadopentetate dimeglumine	3 diagnoses out of 87 patients who received GBCA in 1.5 years (2 cases with Omniscan, 1 with Magnevist)	3.4% (total) 2.3% (Omniscan) 1.1% (Magnevist)	Clinical and deep skin biopsy findings	ESRD population No cumulative effect seen
Heinz-Peer et al (Eur J Radiol **2009**)[27]	Gadodiamide Gadopentetate dimeglumine Gadoterate meglumine	6 patients from 367 dialysis patients developed NSF after CE-MRI	1.63 % (confounded, Magnevist was administered to three patients but all three patients also received Omniscan and one patient also Dotarem)	Clinical and deep skin biopsy findings	
Hope et al (Invest Radiol **2009**)[28]	Gadopentetate dimeglumine	84,659 patients with CE-MRI and 530 patients on dialysis received CE-MRI NSF: 1 definite and 1 possible in dialysis group 2 possible in 2,862 CKD patients	0.19 % in patients on dialysis (incidence is based on definite diagnosis of NSF)	Clinical and deep skin biopsy findings in definite case, Clinical findings only in possible NSF patients	
Pieringer (NDT Plus 2008)[36]	Gadodiamide, Gadopentetate dimeglumine	4 cases in 4 years out of 61 patients	6.6% Omniscan and Magnevist)	Not described	Chronic hemodialysis patients

Prince et al (Radiology **2008**)[55]	Gadodiamide, Gadobenate dimeglumine Gadopentetate dimeglumine	0 NSF patients in 8,669 patients with Magnevist enhanced MRI, 14 patients with NSF in 71,441 patients after Omniscan administration, 1 patient with NSF after Multihance administration in 2,785 patients	0% (Magnevist) 0.02% (Omniscan) 0.04% (Multihance, confounded)	Dermatological examination and skin biopsy	None of the patients with NSF received a standard dose.
Shabana et al (AJR **2008**)[40]	Gadodiamide, Gadopentetate dimeglumine Gadobenate dimeglumine	12 patients with NSF from 414 haemodialysis patients received GBCA.	2.9 % (confounded)	Clinical and deep skin biopsy findings	All together a total of 29 patients developed NSF of which 25 had a documented GBCA administration.
Todd et al. (Arthritis Rheum **2007**)[18]	Gadopentetate dimeglumine	16 patients developed cutaneous changes characteristic for NSF of 54 who received Magnevist enhanced MRI and received haemodialysis.	29.6 %	Cutaneous changes characteristic of NSF	Limitation of the study: NSF diagnosis based on clinical symptoms only, no histopathological examination of affected skin
Wertmann et al (Radiology **2008**)[47]	Gadodiamide, Gadopentetate dimeglumine	4 NSF patients in 135,347 patients who received Magnevist 32 patients of 82,260 developed NSF after gadodiamide administration	0.003% (Magnevist) 0.039 % (Omniscan)	Clinical and deep skin biopsy findings	Incidence was evaluated based on all patients that received GBCA, including patients with normal kidney function.

Deo et al.[25] retrospectively analyzed data from a population of patients with ESRD who were receiving hemodialysis or peritoneal dialysis at one of four dialysis facilities in the US for the

18 month period ending on Jul 01, 2006. Within this population, 87 patients with ESRD had 123 gadolinium-enhanced examinations during the study period, and 3 cases of NSF were diagnosed in the 18 month period ending on Jul 01, 2006 (2 with Omniscan and 1 with Magnevist). The authors reported that their population-based study documented an incidence of NSF of 4.3 cases per 1000 patient years, and a risk of 3.4% per patient, or 2.4% per gadolinium exposure.

Heinz-Peer et al.[27] retrospectively reviewed radiological records of 552 patients with end-stage renal disease, being on hemodialysis or peritoneal dialysis, to identify patients, who had undergone contrast-enhanced MRI and had developed NSF. 146 dialysis patients had undergone no contrast-enhanced MRI and none of these had developed NSF. 195 of 552 patients had undergone a total of 325 Gd-enhanced MRIs. NSF prevalence was 1.6%. Six patients developed NSF; the diagnosis was either confirmed by skin biopsy or by review of medical and histopathological records. The cumulative dose of GBCAs, history of thrombosis, recent surgery and the combination of HD and PD proved to be significant factors for the development of NSF:

Hope et al.[28] conducted a retrospective study of all patients who underwent contrast-enhanced magnetic MRIs between Jan 01, 2004 and May 31, 2007 in a large organization providing managed care for more than 3.3 million residents. Magnevist was used at the facility. Objective of the study was to determine the prevalence of NSF in patients with chronic kidney disease (CKD) who received Magnevist. The authors referenced Bayer's Global Pharmacovigilance data, in which it was reported that some of the company’s NSF reports were confounded by the administration of other GBCAs. Consequently, they decided to determine the prevalence of NSF in patients who received only Magnevist. They separately studied patients on dialysis and patients with chronic renal failure, who were not undergoing dialysis.

Four methods were used to discover cases of NSF: review of pathology slides, review of coded diagnoses, review of visits to dermatologists and rheumatologists, and surveys of physicians. Results: During the study period 115,252 contrast-enhanced MRIs were

performed, including 676 in 530 patients receiving dialysis (92% on chronic dialysis and 8% on acute dialysis) and 3,423 in 2,862 patients with elevated serum creatinine levels at the time of gadolinium chelate administration.

One dialysis patient had a definite diagnosis of NSF. In 3 additional patients, 1 on chronic dialysis and 2 with CKD, NSF could not be ruled out. The authors concluded that the prevalence of NSF in patients with CKD who received gadopentetate dimeglumine is lower than previously reported in patients who have received less stable formulations of gadolinium chelates.

Limitations of the study: Authors stated that the prevalence of NSF was likely underreported in their patient population as all patients were not individually examined and histology was not available in the majority of patients. Furthermore, the prevalence is likely affected by the lower average dose and frequency of gadolinium chelate administration in this study compared with previous reports in the literature.

Pieringer et al.[36] investigated the use of GBCAs and erythropoietin in hemodialysis patients with and without NSF. Four of 65 dialysis patients developed NSF between 2002 and 2006 at their institution. There were no differences given between the NSF and the control patients regarding age, sex, number of kidney transplantations, cumulative time on hemodialysis, or primary renal disease. In the NSF group, on average, more contrast-enhanced MRIs had been performed, the cumulative dose of contrast agent was higher, and higher doses of erythropoietin were administered. The authors conclude that the findings of their study have to be interpreted cautiously due to the low number of NSF patients, and that further studies to search for (co-)triggers in the development of NSF are strongly warranted.

Prince et al.[55] retrospectively reviewed the medical records from 2 hospitals in an attempt to determine the incidence of NSF and associated risk factors in patients who undergo gadolinium-enhanced magnetic resonance imaging. From Jan 01, 1997 to Jun 30, 2007, 15 cases of biopsy-confirmed NSF in patients who received gadolinium-based contrast media were identified. All patients had an eGFR < 30 mL/min, and 11 had acute renal failure or

acute deterioration of chronic renal failure. Authors found that the incidence of NSF was zero in 74,124 patients who had received a standard GBCA dose, and 15 (0.17%) in 8,997 patients who had received a high dose. The 15 cases occurred in association with gadodiamide (14/71,441 or 0.02%) and gadobenate dimeglumine (1/2785 or 0.04%). None of the 8,669 patients who had received Magnevist developed NSF at these two hospitals.

Shabana, et al.[40] searched a dermatopathology database to identify patients in whom NSF was diagnosed. Twenty-nine patients were found to have had NSF between Nov 15, 1999 and Dec 31, 2006, and 25 of these had received gadolinium-based contrast agents prior to diagnosis. Three gadolinium contrast agents (Magnevist, Omniscan, and Multihance) were used during the study period, but in most cases it was not possible to determine which product(s) the patients had received. Only in 1 case was it known that Magnevist was the product used in closest proximity to the onset of NSF-like symptoms.

A database of patients undergoing long-term hemodialysis was reviewed separately to determine the frequency of NSF among these patients and how many had received gadolinium. NSF developed in 12 (2.9%) of 414 patients undergoing hemodialysis who received gadolinium-based contrast material.

Todd et al.[18] recruited two cohorts of patients receiving hemodialysis at six outpatient centers in the U.S., regardless of gadolinium exposure, underlying renal disease, duration of renal disease, or any other medical parameter, and systematically examined them for cutaneous changes of NSF. Skin changes were defined using a scoring system based on hyperpigmentation, hardening, and tethering of the skin on the extremities. The presence of any two of these three criteria were considered a positive exam. In cohort 1, between 5 and 7 of 30 patients, depending on the examiner, demonstrated skin changes of NSF. In Cohort 2, 25 (13%) of 186 patients demonstrated cutaneous changes of NSF. Gadolinium exposure was ascertained for the patients in cohort 2, and cutaneous changes of NSF were observed in 16 of the 54 patients with prior exposure to gadolinium contrast (Magnevist). This incidence rate is higher than in other studies. It should be noted however that only one patient in Cohort 1 and

4 patients in Cohort 2 had confirming skin biopsies that were consistent with NSF. Confirming skin biopsy information was not available for the other patients.

Wertman et al.[47] discussed the results of a multi-institutional retrospective study to determine the benchmark incidence of NSF associated with the use of different gadolinium-based contrast agents at 4 university tertiary care centers in the US. Of the 4 centers studied, 2 centers (Centers "C" and "D") were identified as using Magnevist during the period of the study (between Jan 2000 and Dec 2006), and the other 2 used gadodiamide.

From the 2 centers identified as using Magnevist, there were a total of 4 patients diagnosed with NSF following Magnevist enhanced MR examinations: 3 at Center C and 1 at Center D. The patient at Center D was the only patient who developed NSF out of 65,000 who had received Magnevist. The patient had CKD stage 5, and developed NSF 4 weeks after his last Magnevist administration.

Overall, in the 4 institutions, the incidence was 1 in 2,913 patients who underwent gadodiamide-enhanced MR examinations and 1 in 44,224 patients who underwent Magnevist-enhanced MR examinations. The benchmark incidences of NSF were significantly and remarkably lower ($P < .001$) at the 2 centers where gadopentetate dimeglumine was used compared with those at the 2 centers where gadodiamide was used. Authors concluded that the benchmark incidence of NSF was much greater at the 2 centers where gadodiamide was used than at the 2 centers where Magnevist was used.

4.3.2.2 Status update on MRI study

Pursuant to the FDA request for a post-marketing commitment to conduct studies with all marketed GBCAs to assess the magnitude of the potential risk for the development of NSF in patients with moderate to severe renal impairment, Bayer initiated such a study with Magnevist (MRI study, "Prospective non-randomized observational (pharmacoepidemiologic) cohort study (open-label, multicenter) to assess the magnitude of potential risk with the administration of Magnevist Injection in patients with moderate to severe renal impairment for the development of NSF based on diagnostically specific clinical

and histopathologic information"). The study methodology was developed and based on the proposed study design aspects by the FDA, which were communicated in letters to Bayer in May and August 2007 for Magnevist. The goal of the MRI study is to recruit 1,000 patients with moderate to severe renal impairment (600 patients with moderate renal impairment [ie, eGFR of 30 – 59 ml/min/1.73m^2] and 400 patients with severe renal impairment [ie, eGFR of < 30 ml/min/1.73m^2]), who are scheduled to undergo a contrast enhanced MRI with Magnevist. All patients will be followed-up for 2 years to determine if they have developed any signs or symptoms suggestive of NSF.

Study status (as of September 30, 2009)

The study protocol was finalized in February 2008. The first study site was initiated in September 2008, and the first patient enrolled in November 2008. As of Sep 30, 2009, 17 sites are initiated and a total of 51 patients have been enrolled.

Of these 51 patients, 39 patients (36 with moderate and 3 with severe renal insufficiency) received Magnevist and entered the follow-up portion of the study protocol. The other 12 patients either did not receive Magnevist or, after further testing by the central laboratory, did not meet the protocol definition of moderate to severe renal impairment.

Two patients have died of underlying disease, and one has withdrawn consent. As of Sep 30, 2009 the duration of the maximum follow-up period is 10 months.

Amendments to the study protocol

The protocol was amended twice. The table below summarizes the change and rationale for each amendment.

Amendment No.	Released	Change	Rationale for amendment
1	July 2008	Incorporation of clinicopathological definition of NSF developed by an expert working group facilitated by the American College of Radiology (to date unpublished)	Due to a lack of an available recognized definition of NSF at the time the protocol was written, the description of the disease reflected in the original document was based on typical signs and symptoms of NSF reported in the peer-reviewed literature at that time.
2	June 2009	Change to exclusion criteria: Original protocol: Patients who had received any GBCA within 12 months prior to the scheduled Magnevist-administration are excluded. New protocol: Patients who have received any GBCA other than Magnevist within the last 12 months are excluded.	To better reflect the way in which Magnevist is used in current clinical practice and to assure that the risk of Magnevist in current practice is properly assessed.

4.4 Summary of data on clinical usage of Gd-based contrast agents

Any risk estimation based on clinical evidence for a particular Gd-based contrast agent has to take into account that agent's clinical reports as well as all factors which determine the likelihood of that agent to be used in the patient population of interest, such as total usage over time, approved imaging indications (eg, whole-body MRI, CE-MRI in CNS, CE-MRA), year of launch, and clinical settings of use (eg, use in hospitals or private practices).

4.4.1 Cumulative estimated number of total administrations since approval

To illustrate the significant differences in total usage of the different Gd-based contrast agents the number of estimated total administrations worldwide since launch for the various substances is provided in Text Table 13.

Text Table 13: Cumulative estimated number of total administrations of Gd-based contrast agents worldwide since launch

Contrast agent	Total number of administrations worldwide*	Total number of administrations in the US*	Year of first approval
Magnevist	>105 Million administrations	> 50 Million administrations	1988
Omniscan	> 49 Million administrations	> 25 Million administrations	1993
Dotarem	> 17 Million administrations	Not marketed	1994
Prohance	> 15 Million administrations	> 7 Million administrations	1992
Multihance	> 7.5 Million administrations	> 2.5 Million administrations	1998
Gadovist	> 4 Million administrations	Not marketed	2000
Optimark	> 3.5 Million administrations	> 2,5 Million administrations	1999
Eovist	~ 400.000 administrations	< 50.000 administrations	2004
Ablavar	< 100.000 administrations	Not marketed	2005

* BSP estimates on basis of sales data and data provided by Arlington Medical Resources (AMR) Inc.(Status September 2009)

4.4.2 Range of approved indications and dosages

Furthermore, clear differences exist between the various GBCAs in the approved product labelling of imaging indications and corresponding recommended doses. Agents with a narrow spectrum of approved indications are likely to have a lower probability to be used in patients considered to be at risk to develop NSF, ie, patients with severe renal impairment, than agents with a broad range of approved indications. This may result in a lack of clinical evidence (ie, NSF reports) despite a very similar hypothetical risk profile on the basis of physicochemical, pharmacokinetic, and preclinical data. Text Table 14 provides an overview of the approved indications of the marketed GBCAs and their recommended dosages in the US as well as the European Union (EU) to demonstrate the differences (please note that range of approved indications and doses may differ from country to country).

Text Table 14: Approved indications and cumulative doses of marketed Gd-based contrast agents

Contrast agent	Approved Indications & cumulative doses (US)	Approved indications & cumulative doses EU
Magnevist*	CE-MRI of CNS CE-MRI of Head & Neck whole-body CE-MRI Use in children ≧ 2 years 0.1 mmol Gd/kg BW	CE-MRI of CNS (incl. head, neck) Whole-body CE-MRI CE-MRA Use in children, incl. neonates ≦ 0.3 mmol Gd/kg BW
Omniscan*	CE-MRI of CNS Whole-body CE-MRI Use in children ≥ 2 years ≤ 0.3 mmol Gd/kg BW	CE-MRI of CNS Whole-body CE-MRI CE-MRA Use in children ≥ 6 months ≤ 0.3 mmol Gd/kg BW
Dotarem*	(not marketed)	CE-MRI of CNS Whole-body CE-MRI CE-MRA Use in children ≦ 0.3 mmol Gd/kg BW
Prohance*	CE-MRI of CNS / Head & Neck Use in children ≧ 2 years ≦ 0.3 mmol Gd/kg BW	Whole-body CE-MRI Use in children > 6 months ≦ 0.3 mmol Gd/kg BW
Multihance	CE-MRI of CNS 0.1 mmol Gd/kg BW	CE-MRI of CNS CE-MRI of liver, lesion detection CE-MRA 0.05 – 0.1 mmol Gd/kg BW
Gadovist	(not marketed)	CE-MRI of CNS / Brain Perfusion CE-MRI of kidneys and liver CE-MRA ≦ 0.3 mmol Gd/kg BW Use in children ≧ 7 years
Optimark	CE-MRI of CNS CE.MRI of liver ≦ 0.1 mmol Gd/kg BW	CE-MRI of CNS CE-MRI of liver ≦ 0.1 mmol Gd/kg BW Use in children ≧ 2 years
Eovist	CE-MRI of liver, lesion detection + characterization 0.025 mmol Gd/kg BW	CE-MRI of liver, lesion detection + characterization 0.025 mmol Gd/kg BW
Ablavar	CE-MR Angiography 0.03 mmol Gd/kg BW	CE-MRA 0.03 mmol Gd/kg BW

* Initial approval prior to EU-wide harmoniaztion procedures were in place
References: US PIs and EU SPCs. For products marked with a * the German SPCs was used

Magnevist is the Gd-based contrast agent with the highest usage for CE-MRI worldwide and the broadest range of imaging indications. Introduced in 1988 as the first contrast agent for CE-MRI, Magnevist has since then become the clinical standard in many indications. CNS

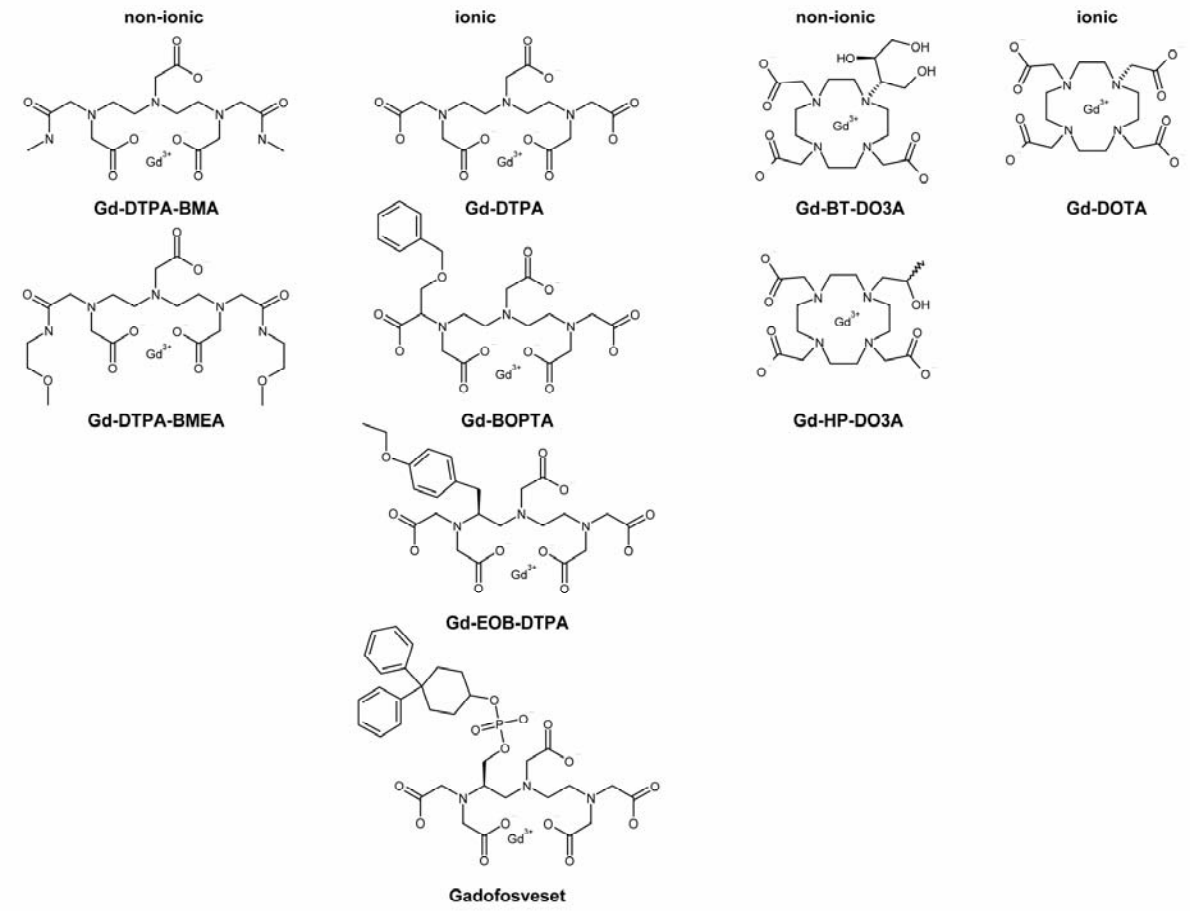
imaging was initially the main area for its use and still remains one major indication for Magnevist-enhanced MRI, but the application range has meanwhile expanded to various indications covering the whole body. The numerous reports in the literature confirm the clinical value as well as favorable efficacy and safety of Magnevist, in particular also including in patients with renal disease, for whom diagnostic alternatives to CE-MRI are in many instances not available.

5. Complex stability of Gd-based contrast agents

5.1 General chemical principles

Based on their chemical structures, Gd-based contrast agents may be divided into 2 major classes: linear and macrocyclic complexes (Text Figure 4). The linear ligands derived from the DTPA backbone wrap around the Gd^{3+} ion and the chelates formed are more flexible as the cages are not fully closed. The ligands of the macrocyclic GBCAs form a rigid cage with a pre-organized cavity to fit the coordination sphere of the Gd^{3+} ion [56 57 58], following structural principles established by Pedersen, Lehn and Cram[59]. From a chemical perspective the two major classes of Gd complexes can be further subdivided into non-ionic and ionic groups. The non-ionic Gd-complexes carry 3 carboxylic acids which are neutralized by the trivalent gadolinium ion. The ionic linear ligands carry 5, the ionic macrocyclic 4 carboxylic acids resulting in negatively charged Gd-complexes.

A: Linear contrast agents ***B: Macrocyclic contrast agents***



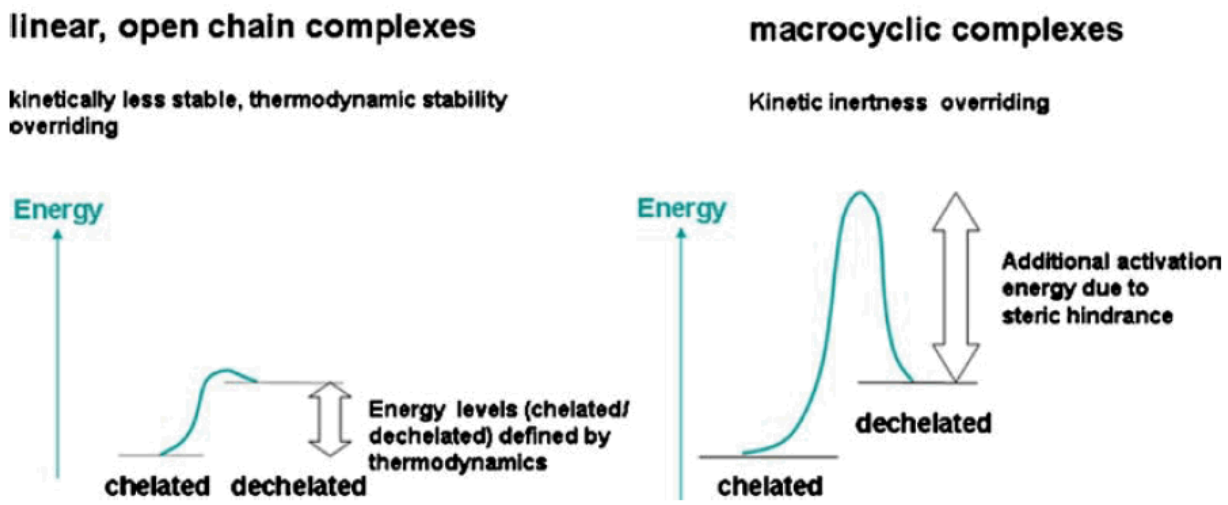
Text Figure 4: Chemical structures of the investigated gadolinium based contrast agents.

Linear Gd-complexes (left columns) are based on the DTPA backbone, while the macrocyclic Gd complexes (right columns) are derived from DOTA. Both classes comprise compounds which are either non-ionic (no net charge) or ionic which are negatively charged.

All GBCAs are characterized by a high complex stability, but there is nonetheless a difference in the potential for dissociation of gadolinium (Gd^{3+}) ions of the Gd-chelate complexes from the 2 groups. This difference may be characterized by 2 distinct parameters:

- kinetic inertia
- thermodynamic stability.

Kinetic inertia is characterized by the dissociation rate. The dissociation rate describes how fast the equilibrium, which is determined by the stability constant, is reached and, thus, how quickly Gd^{3+} is released from the Gd complex. Substantial activation energy is necessary to both generate and dissociate the macrocyclic Gd-complexes (Text Figure 5), and, therefore, under the same conditions, macrocyclic GBCAs have a much slower dissociation rate than linear GBCAs (Text Table 15).



Text Figure 5: Principles of complex stability

Differences in activation energy required between linear and macrocyclic agents to demonstrate stability/inertness of gadolinium-containing contrast agents.

Text Table 15: Overview of dissociation half-lives (T1/2), determined at different conditions, illustrating the kinetic inertias of GBCAs at pH 1 and at higher pH.

(The values are taken from the cited references or were calculated from the given rate constants. Due to the differing conditions the values are not directly comparable.)

Class	Brand name (Generic name)	Short name	$T_{1/2}$, pH 1	Reference	$T_{1/2}$, pH > 5	Reference
Macro-cyclic	Gadovist (Gadobutrol)	Gd-BT-DO3A	8 h, 24 h (37°C)	63, Data on file at Bayer	65 years (pH 5.3, 25°C)	60
	Prohance (Gadoteridol)	Gd-HP-DO3A	2.0 h, 3h (37°C)	63, 61	36 years (pH 5.3, 25°C)	16
	Dotarem (Gadoterate meglumine)	Gd-DOTA	26.4 h, 9 h, 60 h (37°C)	63, 61, 62	37 years (pH 5.3, 37°C)	60

As a comparison: Linear GBCAs: t1/2;: < 5 s, (25°C) 63, pH > 5, 5-7 days[64]

In order to understand the importance of the dissociation rate for the in vivo stability of Gd complexes in a clinical setting, the elimination rates of the respective Gd complexes from the body must be considered. If the dissociation rate is much slower than the elimination rate, any release of Gd^{3+} during the residence time of the Gd-complex in the body should be negligible regardless of its level of stability as expressed by the stability constant.[65]

The complex stability constant, K_{therm} (thermodynamic stability), describes the stability of the deprotonated Gd complex.[66] At physiological pH 7.4 partial protonation of the ligand competes with the complexation of Gd^{3+} reducing the stability of the Gd complex. This is reflected by the lower conditional stability constant, K_{cond}, 7.4. The complex stability is enhanced by the number of charged carboxylates in the coordination sphere of the Gd^{3+}. Each negatively charged oxygen atom binds more strongly to the cation Gd^{3+}, thereby achieving higher thermodynamic stability than an uncharged amide or alcoholic oxygen.[67] This is clearly reflected by the stability constants which are, in general, several orders of magnitude lower for non-ionic than for ionic chelates from the same class (for more details see section 5.2 and Text Table 16).

5.2 Complex stability of linear GBCAs

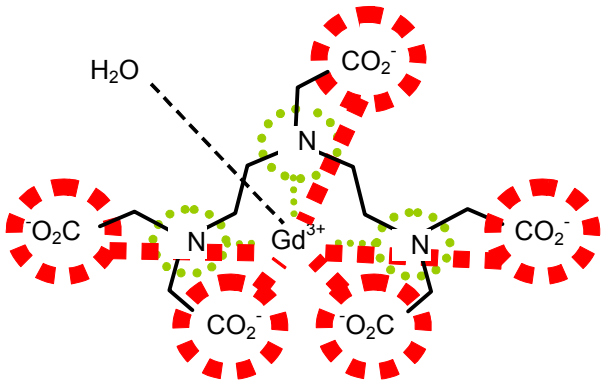
Linear (open-chain) chelates are characterized by thermodynamic (log K_{therm}, valid at pH 14) and conditional complex stability (log K_{cond}, calculated for pH 7.4 with use of the protonation constants of the ligand). The thermodynamic stability constants describe the equilibrium between concentrations of the Gd-complex (ML) on one hand and concentrations of free Gd^{3+} (M) and free ligand (L) on the other hand.

$[M] + [L] \Leftrightarrow [ML]$

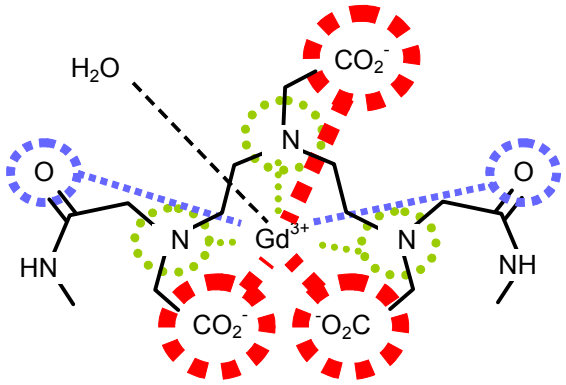
$K = [ML] / [M] + [L]$

The constants are influenced by the charge status of the ligand, ie, an ionic ligand with more than three negative charges (leading to an ionic Gd chelate such as Magnevist) binds stronger to the cation Gd^{3+}, thereby achieving higher complex stability (Text Figure 6), than a ligand with a lower number of negative charges, which results in a neutral chelate (such as Omniscan) upon binding to the Gd^{3+} ion (Text Figure 7).

Text Figure 6: Ionic GBCA



Text Figure 7: Non-ionic GBCA



Furthermore, in a closed environment an increase in the concentration of the free ligand in the formulation of Gd-based contrast agents results in a reduction of the concentration of free Gd^{3+}, particularly for the non-ionic linear agents, as the equilibrium between the concentrations of the Gd-chelate complex and the concentrations of the individual complex partners is shifted to the side of the Gd-chelate to maintain the equilibrium described by the stability constant. Under in vivo conditions, the Gd- complex is surrounded by a variety of competitors, which have the potential to interact with either the Gd^{3+}or the ligand. Proteins, inorganic ions such as phosphate or small organic ligands such as citrate are potent acceptors for Gd^{3+}, whereas other metal ions (eg, zinc) may displace Gd^{3+} from its chelate.
The competition for the Gd^{3} -ion and its ligand may destabilize the Gd-complex in biologic fluids and shift the dissociation equilibrium toward its free components, which most likely do not exist as free ions but bind to other partners which are available in serum or extracellular fluids. The term transmetallation is widely used to designate this exchange process. Text Table 16 displays the respective values of charge, thermodynamic stability, conditional stability, and amount of excess free ligand in the formulation of various linear Gd-chelates.

Text Table 16: Physicochemical properties of linear chelates

Contrast agent *	Charge	Thermodynamic stability (log K_{therm}, pH 14)	Conditional stability (log K_{cond}, pH 7.4)	Excess free ligand in formulation
Omniscan (Gadodiamide)	Non-ionic	16.9	14.9	12 mg/ml (5%)
Optimark (Gadoversetamide)	Non-ionic	16.8	15.0	28.4 mg/ml (10%)
Magnevist (Gadopentetate dimeglumine)	Ionic	22.5	18.4	0.4 mg/ml (≈ 0.1%)
Multihance (Gadobenate dimeglumine)	Ionic	22.6	18.4	0 mg/ml(0%)
Eovist (Gadoxetic acid disodium)	Ionic	23.5	18.7	1.0 mg/ml (≈ 0.6%)
Ablavar (Gadofosveset trisodium)	Ionic	22.1	18.9	0.27 mg/ml (≈ 0.1%)

References: Cacheris 68; White 67; Schmitt-Willich 69 Uggeri 70; Caravan 71; Shellock 72
* not all contrast agents are approved and marketed in all countries worldwide

All GBCAs are very stable compounds, however, among them non-ionic linear chelates possess lower thermodynamic and conditional stability values than ionic linear chelates and are therefore formulated with a comparably high concentration of excess free ligand in the commercial drug product. The ionic linear chelates are characterized by a higher complex stability and thus use lower concentrations of excess free ligand in the commercial formulations.

5.3 Complex stability of macrocyclic compounds

GBCAs such as Dotarem (gadoterate meglumine), Gadovist (gadobutrol), and Prohance (gadoteridol) belong to the group of macrocyclic chelates, which differ from linear chelates regarding the kinetics of complexation and decomplexation. All macrocyclic chelates have a

very high kinetic stability (ie, very high dissociation half-life), compared to that of all linear GBCAs, including that of Magnevist (gadopentetate dimeglumine), Multihance (gadobenate dimeglumine), Eovist (gadoxetic acid disodium), and Ablavar (gadofosveset trisodium). As previously described, the very slow dissociation rates of all macrocyclic GBCAs theoretically result in a negligible release of Gd^{3+} during the residence time of these Gd-complexes in the body, irrespective of their stability constants. Despite the fact that some linear GBCAs have a higher conditional and/or thermodynamic stability than some macrocyclic GBCAs, the kinetic inertia of the macrocyclic GBCAs as described by the dissociation half-life results in a much slower dissociation rate vis-à-vis the linear GBCAs.

5.4 Complex stability of GBCAs in human serum at 37°C

As mentioned previously the GBCAs encounter a variety of competitors under physiological conditions, which have the potential to interact with either the Gd^{3+} or the ligand. Proteins, inorganic ions or small organic ligands are potent acceptors for Gd^{3+}, whereas other metal ions may displace Gd^{3+} from its chelate.[73]

Many attempts have been made to assess the stabilities of GBCAs under physiological conditions, either by using artificial compositions which mimic biologic fluids or by mathematical simulation. However, these approaches insufficiently reflect the in vivo situation, as not all relevant parameters are sufficiently well known for reconstitution or simulation to be exact.

Therefore, Bayer investigated the rate of Gd^{3+} release for all GBCAs commercially available in Europe and/or the US in human serum obtained from healthy subjects.[73] To differentiate between released and complex-bound gadolinium a highly sensitive HPLC-ICP method was used. The influence of elevated phosphate concentrations on complex stability was also investigated. High phosphate levels are often observed in patients with end-stage renal disease, which is the population at risk for NSF.

The results of this in vitro experiment demonstrated that

- Both under physiological conditions and in the presence of elevated phosphate levels all three macrocyclic GBCAs appeared stable for 15 days in human serum at 37°C
- The release of Gd^{3+} after 15 days from the non-ionic linear GBCAs was about 10 times higher than that from the ionic linear GBCAs
- No relevant differences regarding the release of Gd^{3+} were detectable between the ionic linear GBCAs Magnevist and Multihance
- Under physiological conditions, all GBCAs can be divided in principal into 3 distinct stability classes, ie, the non-ionic linear, ionic linear, and macrocyclic GBCAs.

5.5 Summary

Based on all available information on complex stability of the various Gd-chelates, it can be concluded that

- The non-ionic linear chelates (eg, Omniscan, Optimark) have a higher propensity to release Gd^{3+} than either the ionic linear chelates (eg, Magnevist, Multihance) or the macrocyclic chelates (Dotarem, Gadovist, Prohance).
- The risk of Gd^{3+} release with Magnevist does not differ from that of other ionic linear Gd-chelates (including Multihance) based on the available information discussed above.
- All macrocyclic GBCAs demonstrate a very high kinetic stability (comparable among all macrocyclic GBCAs), which suggest a lower potential for these GBCAs to release Gd^{3+} ions in vivo in comparison to both ionic and non-ionic linear Gd-chelates.

6. Pharmacokinetic properties of Gd-based contrast agents

6.1 General principles

Prolonged retention of Gd-based contrast agents in patients with renal impairment may increase the potential that Gd^{3+} ions may be released from the Gd-ligand complexes. Therefore, the in vivo pharmacokinetics (in particular available elimination pathways and serum elimination half life [t ½]) of the Gd-chelates are an important factor to consider in the overall risk assessment regarding the possible association between a particular GBCA and NSF.

For almost all the currently marketed Gd-based contrast agents, including Magnevist, the primary elimination pathway is the kidney. Only Eovist has two equally effective elimination pathways via kidney and bile, which lead to faster elimination and higher clearance, resulting in a lower overall systemic exposure (AUC).

The serum elimination half-life for all contrast agents can be prolonged in patients with renal impairment. Dedicated studies to investigate the safety, tolerability and pharmacokinetics in patients with different degrees of renal impairment were performed for many of the marketed Gd-contrast agents including Magnevist and Eovist.

A summary of the available data on serum elimination pathways and half-lives for various GBCAs in healthy volunteers and patients with renal impairment is displayed in Text Table 17.

Text Table 17: Pharmacokinetic properties of GBCAs in healthy subjects and patients with renal impairment of GBCAs

Contrast agent*	Protein binding	Elimination pathway	Serum elimination half-life Healthy vol.	Serum elimination half-life Mod. Ren. Imp.	Serum elimination half-life Sev. Ren. Imp.
Magnevist (Gadopentetate dimeglumine)	None	Kidney	90 min	4-10 h[74]	< 30 h[74]
Omniscan (Gadodiamide)	None	Kidney	ca. 70 min		
Dotarem (Gadoterate meglumine)	None	Kidney	96 min		
Prohance (Gadoteridol)	None	Kidney	96 min		
Multihance (Gadobenate dimeglumine)	< 5%	Kidney ≧ 96% Bile < 4 %	72 – 102 min	6 h[75]	10-42 h[75][76]
Gadovist (Gadobutrol)	None	Kidney	78 - 126 min	5.5-7.5 h[77]	18-20h[77]
Optimark (Gadoversetamide)	None	Kidney	103 min	7-9 h[78]	
Eovist (Gadoxetic acid disodium)	< 10%	Kidney 50% Bile 50%	60 min	2 h[79]	20 h[79]
Ablavar (Gadofosveset trisodium)	> 85%	Kidney ≧ 91% Bile < 9%	18.5 h	50 h[80]	70 h[80]

* not all contrast agents are approved and marketed in all countries worldwide
References: US or European Package Inserts

Based on summary Text Table 17, it can be concluded that:

- The serum elimination half-life for all GBCAs, which are predominantly eliminated via the kidneys and regardless of their chemical structure, increases as the level of renal impairment increases.

- The majority of the currently marketed extracellular GBCAs (ie, Omniscan, Optimark, Magnevist, Multihance, Dotarem, Gadovist, Prohance) are predominantly eliminated via the kidneys with comparable serum elimination half-lives both in healthy subjects and patients with renal impairment

- Eovist exhibits the highest overall clearance, which leads to the shortest serum elimination half-life in healthy volunteers as compared to other available GBCAs currently marketed, due to two equally effective elimination pathways via kidney and bile.

- The serum elimination half-life of Ablavar is substantially longer in healthy volunteers than that of all other available GBCAs currently marketed, which is primarily the result of the high level of transient protein (albumin)-binding in human serum.

In patients with end-stage renal disease requiring hemodialysis the serum elimination half-life is dependent on the dialysability of the GBCAs administered as well as the timing of the dialysis sessions following the administrations of these agents. Dedicated studies to investigate the pharmacokinetics in patients with end-stage renal failure and dialysability were performed for some of the marketed Gd-contrast agents, including Magnevist[81] [82] and Eovist.

In summary, all Gd-based contrast agents may be removed from the body by hemodialysis. At least 98% of the administered dose of extracellular Gd-based contrast media such as Magnevist is eliminated from the plasma following three consecutive sessions of hemodialysis.

6.2 Pharmacokinetic properties of Magnevist

The pharmacokinetics of intravenously administered gadopentetate (gadopentetate dimeglumine) in normal subjects conforms to a two compartment open-model with mean distribution and elimination half-lives (reported as mean ± SD) of about 0.2 ± 0.13 hours and 1.6 ± 0.13 hours, respectively. Gadopentetate is predominantly eliminated in the urine with 83 ± 14% (mean ± SD) of the dose excreted within 6 hours and 91 ± 13% (mean ± SD) by 24 hours, post-injection. There was no detectable biotransformation or decomposition of gadopentetate dimeglumine.

The renal and plasma clearance rates (1.76 ± 0.39 mL/min/kg and 1.94 ± 0.28 mL/min/kg, respectively) of gadopentetate are essentially identical, indicating no alteration in elimination kinetics on passage through the kidneys and that the drug is essentially cleared through the kidney. The volume of distribution (266 ± 43 mL/kg) is equal to that of extracellular water and clearance is similar to that of substances which are subject to glomerular filtration.

In vitro laboratory results indicate that gadopentetate does not bind to human plasma protein. In vivo protein binding studies have not been done but it is likely, that gadopentetate behaves here like other GBCAs with similiar properties.

In patients with moderate hepatic impairment, the mean distribution and elimination half-lives of Gadopentetate were comparable to healthy matched controls. The mean total CL and renal CL of Gadopentetate were identical in subjects with moderate hepatic impairment and healthy matched controls. The mean V_{ss} in subjects with moderate hepatic impairment was similar to that of healthy matched controls. Mean cumulative excretion (% dose) at 48 hours for subjects with moderate hepatic impairment was similar to that for the healthy matched controls. The mean C_{max} of Gadopentetate in subjects with moderate hepatic impairment was almost the same as that for healthy matched controls. The mean AUC of Gadopentetate for subjects with moderate hepatic impairment was similar to that for the healthy matched controls.

The pharmacokinetic characteristics of Gadopentetate were comparable between healthy non-elderly males and females. The systemic clearance and renal clearance of Gadopentetate were lower in healthy elderly subjects as compared to healthy non-elderly subjects, as expected due to their physiological condition. However, the Gadopentetate urinary recovery at 48 hours in the healthy non-elderly and healthy elderly subjects (males) was similar.

Results of a clinical study with Magnevist demonstrated that in patients with impaired renal function the serum elimination half-life of Magnevist was prolonged, with values of 1.5 to 2 hours for patients with a GFR of 60 to 80 ml/min and values of approximately 4 hours and 10 hours for patients with a GFR of 40 to 60 ml/min and 20 to 40 ml/min respectively[74]. In

patients with end-stage renal failure requiring hemodialysis the mean elimination half-life increased to up to 30 hours when patients were not subject to hemodialysis. The elimination via the kidneys was complete (up to 100%) and remained the predominant route of elimination.

Seventy patients with end-stage renal disease requiring hemodialysis underwent contrast-enhanced MR examination with Magnevist[83]. After the examination, the patients were hemodialyzed according to the usual schedule of 3 dialysis sessions per week at 4 h each. Average cumulative excretory rates of Magnevist were 78.2%, 95.6%, 98.7% and 99.5% in the first to fourth hemodialysis sessions, respectively.

In summary, with the exception of Eovist and Ablavar no relevant difference between Magnevist and the other Gd-based contrast agents regarding the serum elimination in patients with renal impairment has been detected.

7. Overview of nonclinical studies to elucidate the pathomechanism of NSF performed by BSP

Exploratory nonclinical non-GLP in-vivo studies as well as a nonclinical GLP study were initiated by BSP shortly after the first reports on a possible association between GBCA administration and NSF were published in 2006. The objective of these studies was to investigate this possible association and to evaluate in more detail the potential pathogenesis of this new disease entity. In particular possible differences between different Gd-based contrast agents regarding their potential role as a factor in the onset of NSF were to be evaluated. The study objectives, methods and results for these studies are summarized in the subsequent chapters.

7.1 Pathology peer review of skin histology slides from nonclinical studies for gadopentetate dimeglumine and gadoxetic acid disodium

Prior to the first reports on a possible association between GBCA administration and NSF, the studies that had been undertaken, the clinical experience following marketing approval

and the medical/scientific literature published to that point in time had not indicated that skin was a toxicological target of GBCAs. Despite the fact that these initial clinical reports of a possible association did not involve gadopentetate dimeglumine and gadoxetic acid disodium, BSP nevertheless decided to re-investigate the histology slides from the pre-marketing preclinical studies through a pathology peer review procedure to confirm that there had been no early preclinical signs potentially related to NSF. Pursuant to this procedure, the slides of several GLP repeated dose systemic toxicity studies with gadopentetate dimeglumine or gadoxetic acid disodium administration to rats or dogs, including the recovery groups, were re-inspected by pathologists from BSP, who were not involved in the initial assessment.

For the three GLP studies performed with gadopentetate dimeglumine, the BSP pathologists confirmed that the quality of the histopathological sections and accountability of tissues for examination were adequate for evaluation. For the 4 week rat study the internal re-examination resulted in the conclusion that administration of Magnevist up to 5.0 mmol/kg body weight over 4 weeks caused no treatment related histopathological findings in the skin. The same conclusion was drawn for the 31 week recovery group from male rats treated for 4 weeks with 5.0 mmol/kg and for dogs treated for 4 week with up to 2.5 mmol/kg Magnevist. Thus, all internal re-examinations confirmed the initial assessment in the three repeat dose toxicity studies.

For the two GLP studies performed with gadoxetic acid disodium, the quality of the histopathological sections and accountability of tissues for examination were again confirmed to be adequate for evaluation. No treatment related histopathological findings in the skin were observed, thus the internal re-examinations confirmed the initial assessment in the two repeat dose toxicity studies for gadoxetic acid disodium.

In addition, a Pathology Working Group (PWG) consisting of five independent and internationally acknowledged pathologists was set up and organized by a contract research organisation (CRO) in 2007 to conduct a blinded external peer review of available skin histologies from certain preclinical studies with gadopentetate dimeglumine. Slides from a repeated high dose systemic toxicity study in rats, slides from a 31 week recovery group of

another repeated systemic toxicity study in rats and slides from a newly conducted exploratory non-GLP rat study including Magnevist, gadodiamide, caldiamide (the Ca-chelate of the Omniscan ligand), Omniscan and Gd-EDTA (see also chapter 3.3), were subject to the blinded external peer review by this Pathology Working Group (PWG), which evaluated 122 blinded hematoxylin and eosin stained skin slides.

The members of the PWG independently scored each blinded skin tissue section and then, through discussion, provided a consensus grade for all sections examined during the review. During the assessment, no records were made available as to how the number of animals for each subgroup was selected, whether all the animals were followed to the end of the study, or how many samples originated with each animal (blinded review).

During the PWG's external peer review, a score ranging from 0 (no ulcer or crusts) to 4 (multiple ulcers with inflammatory reactions) was assigned to each skin tissue slide, indicating the severity of the observed lesion. The median severity of the score for each of the compounds examined ranged from 1.0 (NaCI, Magnevist and caldiamide) to 4.0 for gadodiamide. Ulcers were present only in those groups treated with gadodiamide, Gd-EDTA or Omniscan. The median severity for ulcers was 3.5 for gadodiamide, 2.0 for Gd-EDTA and 3.0 for Omniscan. Based on these data, the PWG recognized a treatment related effect for gadodiamide, Gd-EDTA and Omniscan. Further, the PWG's consensus opinion was that the histology results did not indicate a morphological difference for Magnevist or caldiamide when these groups were compared to saline. The PWG, however, cautioned that the predictive power of these findings regarding an association between the administration of GBCAs and NSF is limited by the chosen animal model and the small number of animals involved in the study, and that the PWG, therefore, recommended that the company consider undertaking further studies to attempt to address these limitations.

7.2 Gadolinium-Based Contrast Agents (GBCAs) and Nephrogenic Systemic Fibrosis (NSF): Effect of GBCAs and zinc depletion on occurrence of NSF-like skin lesions in rats

Published in: H. Pietsch et al. [84]

Study objectives: The objective of the study was to evaluate the effect of endogenous zinc (Zn) depletion which might result from the administration of different Gd complexes (Omniscan, Optimark, Magnevist, Gadopentetic acid dimeglumine (drug substance of Magnevist without excess of free ligand), Gadovist, and Gd-EDTA (Gd-complex with very low in-vivo stability) given repeatedly over a period of 5 weeks on the development of skin changes.

An additional objective of the study included the evaluation of the effect of endogenous zinc depletion in addition to the administration of different GBCAs on serum levels of Zn and copper (Cu).

Study description: As no adequate nonclinical animal model for end-stage renal disease (ESRD) exists, rats were injected with high doses of GBCAs to simulate the exposure of patients with severe renal malfunction/ kidney impairment before hemodialysis.

Male rats were injected intravenously with different GBCAs (Omniscan, Optimark, Magnevist, Gadovist, Gadopentetate, Gd-EDTA) over a period of 5 weeks once daily (over 5 consecutive days per week) at a high dose into the tail vein. In addition half of the rats were kept on a zinc-deficient diet for 6 weeks before and during the injection of contrast agents and were injected intravenously with the same contrast agents and dosing regime.

Body weight and macroscopic changes of the skin were recorded at the time of each injection. At the end of the experiment (5 days after the last injection, ie, on day 40) gadolinium, zinc and copper concentrations in tissues (ie, skin, femur, liver, kidneys, spleen, lung, heart, mucle) and serum were measured, organ weights were determined, and a histolopathological evaluation of the skin specimens was performed.

Results: The most extensive skin lesions could be observed in the animals treated with Gd-EDTA. The observed skin lesions included erythema, multifocal ulcerations, multiple crusts, increased dermal cellularity and dermal fibrosis. High Gd concentrations in the skin were observed in these animals.

ln 2 of 12 animals treated with Omniscan, slight histopathological changes were observed, ie, development of crust in one animal fed with Zn depletion diet and of acanthosis in one animal fed with standard diet, also in 1 of 11 animals treated with gadopentetate slight histopathological changes were observed. In the animals treated with Omniscan, high Gd concentrations in the skin were obtained. Much lower Gd levels were observed in the animals treated with gadopentetate, which were comparable to those obtained in animals treated with Magnevist. (With the method used for Gd detection, it was not possible to distinguish between bound and unbound Gd.)

No effect on skin was observed in any of the treated animals following the administration of Magnevist, Gadovist and Optimark.

Five days after the last administration of the Gd-based substances, Gd was detected in all animals not only in the skin, but also in liver, femur, heart, lung, spleen, muscle, and kidney. Values differed among the various contrast agents and across tissues.

The highest Gd concentrations were observed in tissue specimens of all organs (except the kidney) obtained from animals treated with Gd-EDTA. For this compound multiples of the values obtained in all other treatment groups were observed in many tissues.

Among the treatment groups with the marketed contrast agents, higher Gd levels in skin, liver and femur were obtained in animals treated with Omniscan than in animals treated with Optimark, Magnevist and Gadovist. In all other tissue specimens no relevant differences in Gd levels were observed between these contrast agents.

No significant effect of endogenous Zn-depletion (ie, low serum levels of Zn) could be detected regarding the macroscopic and microscopic evaluation of the skin specimens as well as Gd levels in the different tissue specimens.

No differences between the various treatment groups were observed for serum and tissue concentrations of the endogenous ion Cu, regardless of the endogenous serum Zn level.

Conclusions: None of the rats treated with Optimark, Magnevist, Gadovist*, Omniscan and gadopentetate showed any kind of macroscopically detectable skin lesions during or at the end of the study. The most extensive NSF-like skin lesions could be observed in the animals treated with Gd-EDTA as well as in all animals, which erroneously received a single injection of gadodiamide (drug substance of Omniscan without excess of free ligand) in addition to 24 injections of Omniscan. High Gd concentrations in the skin were observed in the animals treated with Gd-EDTA and gadodiamide/Omniscan.

Slight histopathological changes were also observed in single animals treated with Omniscan (2 of 12 animals) and gadopentetate (1 of 11 animals). No clear effect of the endogenous Zn depletion in half of the animals as a result of the Zn-depleted diet used could be detected regarding the occurrence of any skin changes or Gd concentrations in the different tissue specimens. No differences between the different treatment groups were observed for serum and tissue concentrations of the endogenous trace metal Cu.

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* Calcinosis cutis, which was observed in a single animal treated with Gadovist, is considered to be an unspecific finding not directly associated with the administration of Gd.

7.3 Gadolinium-Based Contrast Agents (GBCAs) and Nephrogenic Systemic Fibrosis (NSF): Effect of GBCAs (Gadodiamide, Omniscan, Magnevist) on endogenous trace metal levels (Ca2+, Zn2+ , Cu2+ and Mg2+) and occurrence of NSF-like skin lesions in rats and role of subcutaneous zinc supplementation

Published in: H. Pietsch et al.[85]

Study objectives: The objective of the study was to evaluate the role of different Gadolinium-based contrast agents (GBCA; ie, Magnevist, Omniscan, gadodiamide (Gadopentetate-BMA, drug substance of Omniscan without an excess of free ligand)) given repeatedly over a period of 4 weeks in the pathogenesis of skin changes.

Additional objectives of the study included

- The evaluation of the effect of subcutaneous (s.c.) zinc (Zn) prophylaxis on pathogenesis of skin changes and
- The evaluation of the effect of Gd-based contrast agents on endogenous trace metal ion in the skin (Zn) and in the serum (Zn, Magnesium (Mg), Calcium (Ca), and Cu).

Study description: As no adequate nonclinical animal model for ESRD exists, rats were repeatedly injected with high doses of GBCAs to simulate the exposure of patients with severe renal malfunction/ kidney impairment to GBCAs before hemodialysis.

Rats were injected intravenously with different GBCAs (Magnevist, and Omniscan), Gadodiamide (the drug substance of Omniscan without excess ligand) and saline over a period of 4 weeks once daily (over 5 consecutive days per week) at a high dose into the tail vein. In addition, half of the rats received subcutaneous (i.c.) zinc-aspartate for zinc supplementation.

Body weight and macroscopic changes of the skin were recorded at each injection time point. At the end of the experiment (5 days after the last injection) Gd and Zn concentrations in tissues (ie, skin, femur, liver, kidneys) and Zn, Cu, magnesium (Mg) and calcium (Ca) serum levels were measured. Organ weights were determined and a histopathological evaluation of

skin specimens was performed. The chosen analytical method is not able to distinguish between bound and unbound Gd.

Results: The most extensive macroscopic skin lesions were observed in animals treated with gadodiamide, which developed erythema, multifocal ulcerations, multiple crusts, increased dermal cellularity and dermal fibrosis. Very high Gd concentrations (1500 nmol Gd/g skin) in the skin were observed in these animals.

Similar changes, but to a lower incidence and severity, were observed in the animals treated with Omniscan. In these animals, also very high Gd concentrations in the skin were observed, which were in the same range as the values in the gadodiamide group.

No macroscopic or microscopic effect on skin was observed in any of the treated animals following the administration of Magnevist and 0.9% saline. Only low Gd concentrations (< 200 nmol Gd/g skin) were observed after the Magnevist treatment.

CD34 positive mesenchymal spindle-shaped cells (most likely fibroblasts) were present in clusters preferentially in the superficial dermis in animals treated with gadodiamide and to a lesser extent in animals treated with Omniscan. No CD34 positive mesenchymal spindle-shaped cells were observed in the Magnevist group.

Macroscopically, no effect of Zn supplementation was detectable in the skin. Microscopic analyses revealed a slightly lower incidence and lower severity of the findings in the Zn supplementation groups treated with Omniscan in comparison to the respective groups on control diet.

Five days after the last administration of Gd-based substances, Gd was detected in all animals not only in the skin, but also in liver and femur. Values differed among the various contrast agents and across tissues.

Conclusions: No effect on skin was observed in any of the treated animals following the administration of Magnevist and 0.9% saline. The most extensive skin lesions could be

observed in the animals treated with gadodiamide. Similar changes, but to a lower incidence and severity, were observed in the animals treated with Omniscan.

The highest Gd concentrations in the skin, liver and femur were observed after the administration of gadodiamide followed by the administration of Omniscan. After administration of Magnevist overall much lower Gd levels were detected in the different body tissues, especially in the skin and in the femur. No Gd could be detected after the administration of saline.

A correlation between very high gadolinium content in the skin and the occurrence of NSF-like skin lesions in these animals was observed. No loss of endogenous trace metal ions could be detected following administration of GBCAs. Zinc supplementation resulted in a slightly lower incidence and severity of observed microscopic skin changes.

7.4 Gadolinium-Based Contrast Agents (GBCAs) and Nephrogenic Systemic Fibrosis (NSF): Effect of GBCAs on occurrence of NSF-like skin lesions in rats

Published in: MA Sieber et al. [86], MA Sieber et al.[87], MA Sieber et al. [88]

Study objectives: The objective of the study was to verify whether multiple intravenous administrations of GBCAs used for MRI can induce NSF-like lesions in rats and to evaluate the gadolinium concentration in various tissues after application of GBCAs *in vivo*. Another objective was to investigate the role of the excess ligand on the development of NSF-like lesions and the Gd-concentration in tissues.

Study description: As no adequate nonclinical animal model for ESRD exists, rats were repeatedly injected with high doses of GBCAs to simulate the exposure of patients with severe renal dysfunction/ kidney impairment to GBCAs.

Groups of 6 male rats each were injected intravenously with different GBCAs (Omniscan, Optimark, Magnevist, Multihance, Gadovist, Dotarem, Ablavar and Eovist), GD-EDTA, caldiamide (the Ca-chelate used in Omniscan) and saline over a period of 4 weeks once daily (over 5 consecutive days per week) into the tail vein. The administered dose was 2.5 mmol/kg

for the GBCAs, except for Ablavar and Eovist, which were applied at 1.0 mmol/kg. Gd-EDTA was administered at two doses (0.05 and 0.1 mmol/kg), as was caldiamide (0.5 and 2.5 mmol/kg).

In addition formulations of gadodiamide and gadoversetamide with no and with 5% (gadoversetamide) or 10% (gadodiamide) amounts of excess ligand were tested at doses of 0.5 (gadodiamide only) and 2.5 mmol/kg.

Body weight and macroscopic changes of the skin were recorded at each time of injection. At the end of the experiment (5 days after the last injection) gadolinium, Zn and copper (Cu) concentration in various tissues and serum levels were measured. Organ weights were determined and histopathological evaluation of the skin was performed.

Results: Whereas both macroscopic (in 7 of 12 animals) and microscopic (in 8 of 12 animals) skin lesions were observed in Omniscan-treated animals, no effects (neither macroscopic nor microscopic) on the skin were observed in any of the animals treated with Magnevist, Multihance, Eovist, Gadovist, Dotarem, and Ablavar*. Following the treatment with Optimark, mild microscopical changes were observed in only 1 of 12 treated animals.

For the other tested compounds the most extensive skin lesions (both macroscopic and microscopic changes) were observed in the animals treated with gadodiamide, gadoversetamide, and Gd-EDTA at high doses (2.5mmol/kg and 0.1mmol/kg, respectively). After administration of gadodiamide and Gd-EDTA at low doses (0.5 mmol/kg and 0.05 mmol/kg, respectively) no macroscopic skin lesions were observed in any of the treated animals and microscopic skin lesions were observed to a lesser extent in all animals treated with the low dose of gadodiamide.

* It has to be noted that the observed calcinosis cutis is not considered to be a treatment related finding in the skin since it has also been observed in animals not treated with any Gd-based compounds. The cause of this unspecific lesions remains unknown, but could be related to differences in animal handling (micro-traumata with calcium release) or may be due to differences in the susceptibility of individual animals to Vitamin D and/or Ca in the diet.

Treatment with gadodiamide and gadoversetamide in formulations with 10% excess free ligand did not result in any macroscopic skin changes in any of the treated animals. Microscopic skin changes were only observed in 1 of all treated animals following the administration of Optimark (standard formulation of gadoversetamide with 10% excess free ligand). No skin changes were observed in any of the animals treated with (Gd-free) caldiamide regardless of the dose used.

Five days after the last administration of the different Gd-based substances, Gd was detected in all animals not only in the skin, but also in liver and femur. Values differed among the various contrast agents and across tissues with the most prominent differences observed in skin and femur. It has to be noted that it was not possible to differentiate between chelated and unchelated Gd with the measurement method used.

Among the marketed Gd-based contrast agents, the highest Gd levels in the skin were observed after the treatment with Omniscan (1697.3 ± 244.1 nmol Gd/ g tissue). Lower Gd concentrations were obtained in Optimark-treated animals (428.7 ± 85.9 nmol Gd/ g tissue), but these were still considerably higher than the values obtained after treatment with all other marketed products. Intermediate concentrations were observed after treatment with Magnevist (184.0 ± 78.4 nmol Gd/ g tissue), Ablavar (109.8 ± 19.6 nmol Gd/ g tissue) and Multihance (82.5 ± 10.5 nmol Gd/ g tissue). The lowest Gd concentrations were observed after Gadovist (49.1 ± 8.8 nmol Gd/ g tissue), Dotarem (51.1± 11.5 nmol Gd/ g tissue) and Eovist (10.0 ± 2.8 nmol Gd/ g tissue) treatment.

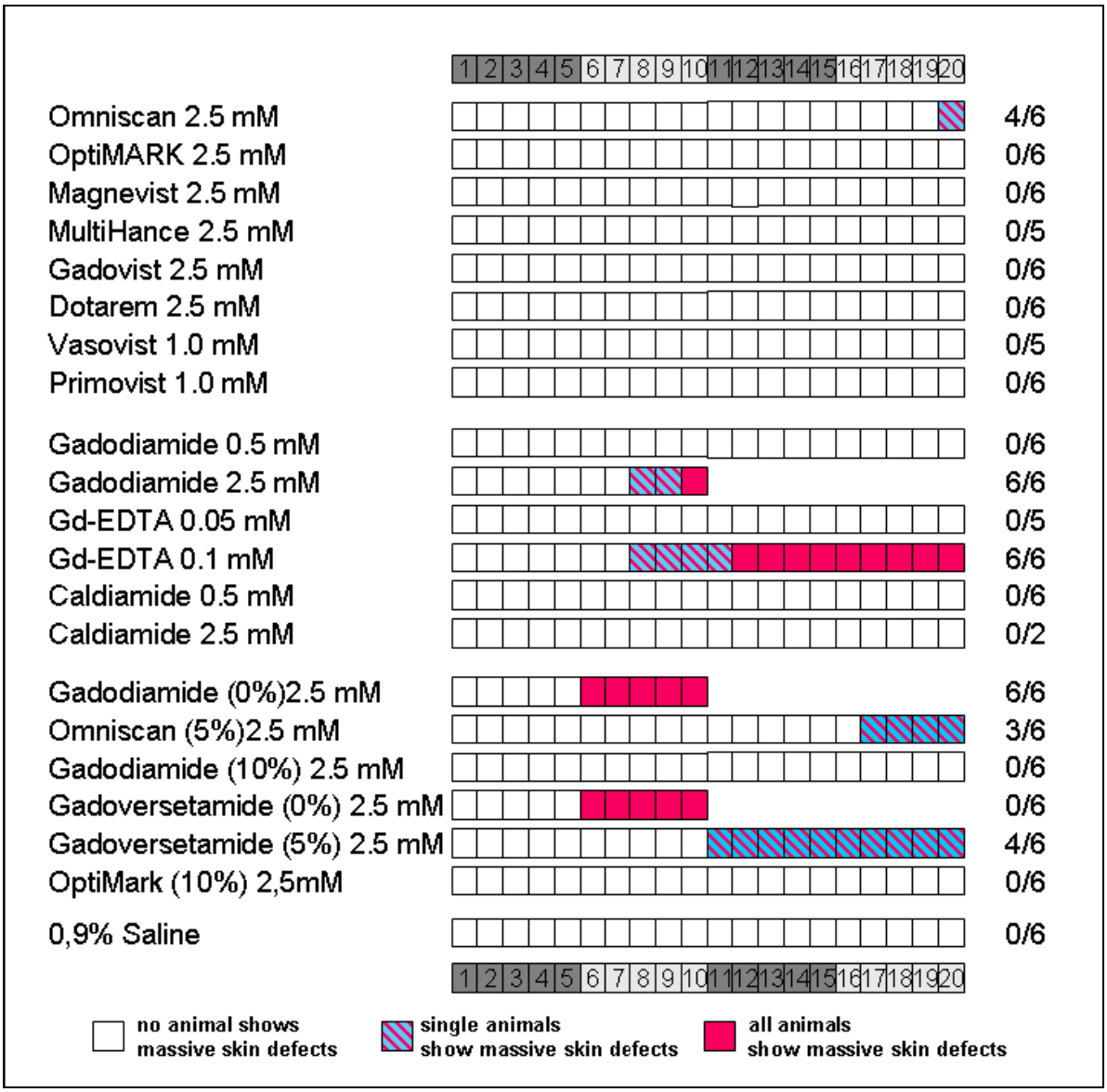
The highest Gd concentrations in the skin were found for the other tested compounds, namely after the treatment with gadodiamide (at high dose), gadoversetamide, and Gd-EDTA (at high dose). The corresponding lower Gd concentrations in the skin after the lower doses of both gadodiamide and Gd-EDTA administration suggest a dose-dependency of Gd administration.

An increase of excess free ligand of 10% or 5% in the formulation of gadodiamide and gadoversetamide, respectively resulted in a decrease of Gd-concentrations in the skin of all

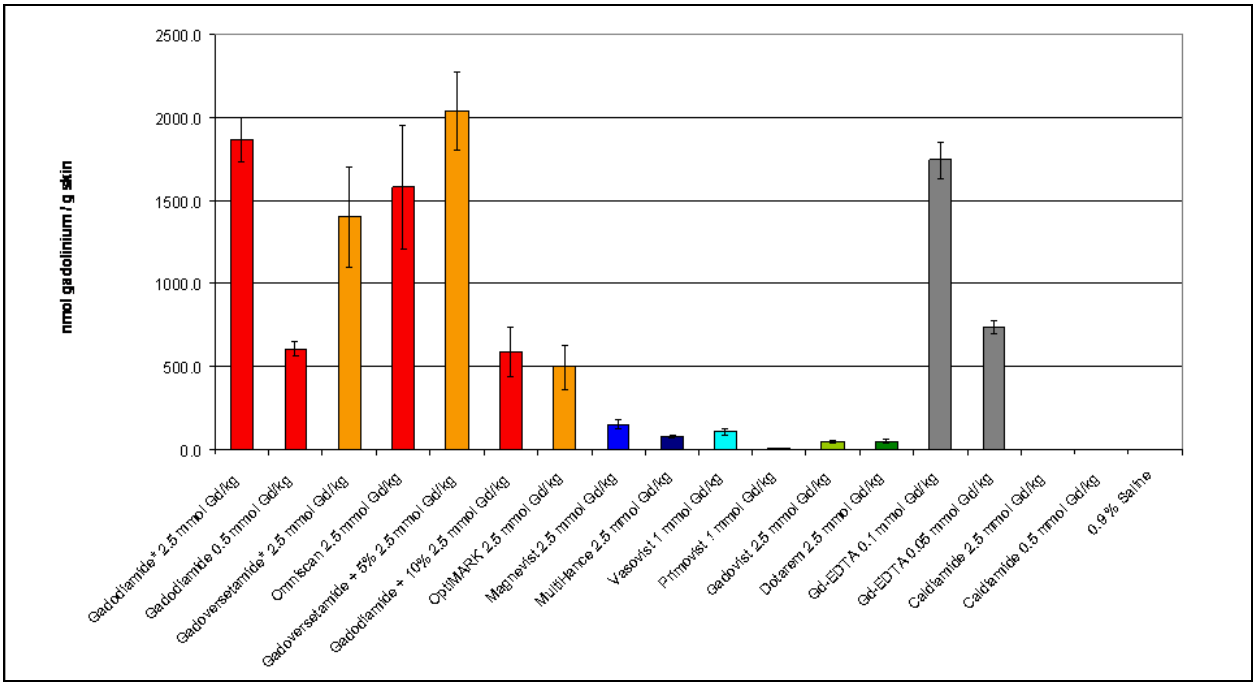
treated animals in comparison to animals treated with gadodiamide and gadoversetamide without any excess free ligand in the formulations.

No Gd was detected in any of the animals treated with caldiamide and saline.

The results of the study are summarized in the following figures.



Text Figure 8: Summary of the macroscopic and microscopic skin findings after treatment of rats with various GBCAs, Gd-based compounds and controls



Text Figure 9: Gadolinium concentrations in skin biopsies from rats treated with various GBCAs, Gd-based compounds and controls (please note that the chosen method of Gd analysis [ICPMS] allows no differentiation between chelated and unchelated Gd).

No significant loss of Cu and Zn was observed in the different treatment groups.

Conclusions: Following the administration of marketed GBCAs macroscopic and microscopic NSF-like skin lesions were only observed in some of the Omniscan-treated animals. No effects were observed in any of the animals treated with Optimark, Magnevist, Multihance, Eovist, Gadovist, Dotarem, and Ablavar. Following the treatment with Optimark, mild microscopical changes were observed in only 1 of 12 treated animals.

Based on results of different doses of gadodiamide and Gd-EDTA, a dose-dependency regarding the occurrence of skin lesions and the accumulation of Gd in skin tissue after the administration of Gd-based compounds may be suggested.

Based on results with gadodiamide and gadoversetamide in formulations with different levels of excess free ligand (ie, 0%, 5%, and 10%), some protective effect of the excess free ligand in the formulation of non-ionic linear chelates on the occurrence of skin changes and the accumulation of Gd in skin tissue can be postulated. An effect of the chelating agent of Omniscan (ie, Caldiamide) itself on the occurrence of skin lesions may be excluded.

Five days after the last administration of the different Gd-based substances, Gd was detected in all animals not only in the skin, but also in liver and femur. Values differed among the various contrast agents and across tissues with the most prominent differences observed in skin and femur. Overall high Gd concentrations in the skin were associated with the occurrence of skin lesions.

Omniscan and Optimark, the marketed non-ionic linear Gd-chelates, showed a higher accumulation in the skin (and to a lesser extent also femur) than all the other marketed products, which may be the result of their low conditional complex stability and higher likelihood of transmetallation.

On the other hand, the very low Gd concentrations in the skin (and also femur) following the administration of Gadovist and Dotarem may be linked to the high kinetic complex stability of these compounds resulting from the macrocyclic structure and very low Gd exchange rate.

The lowest Gd concentrations in skin and femur were determined in Eovist-treated animals, which is most likely attributable both to the lower total dose of Gd administered to the animals as well as the dual elimination pathway in rats and humans of Eovist via kidneys (50%) and liver (50%).

7.5 Potential long time retention of Gadolinium based contrast agents after intravenous administration in rats

Published in: H Pietsch et al. [89]

Study objectives: The objective of this study was to determine the elimination time course of Gd in skin tissue and the potential long-term retention of Gd in skin tissue after the i.v. administration of different marketed GBCAs.

Study description: 6 animals per group were selected at random and the animals were treated in 3 experimental sets with the following compounds: Omniscan, Magnevist, Gadovist (set 1), Multihance, Prohance, Dotarem (set 2), and Optimark (set 3) respectively. There were 2 negative control groups: untreated (set 1) and saline treated animals (set 3). The GBCAs and saline were injected into a tail vein once daily on five consecutive days at a dose of 2.5 mmol Gd/kg body weight. Animals were inspected daily for macroscopic skin changes. Skin biopsies were taken at various time points up to 365 days post-injection.

The gadolinium concentration in the skin samples was determined by Inductive Coupled Plasma Mass Spectrometry (ICP-MS 7500a, Agilent, Waldbronn, Gemany) by measuring the most abundant isotope 158Gd. (It should be noted that this method can not distinguish between chelated and unchelated gadolinium.)

Results: No macroscopic skin changes were observed in any treatment group during the entire course of the experiment.

For several weeks after the last injection the gadolinium concentration in the skin substantially decreased in the animals of all treatment groups. About 60 days after treatment with linear compounds a plateau phase for the gadolinium concentration was observed, where after the gadolinium level decreased only marginally during the next 304 days.

Gadolinium was detected up to one year after the last Gd injection of linear GBCAs with the highest values observed after treatment with the non-ionic linear compound Omniscan. At day 364 p.i. highest Gd values were observed after treatment with the non-ionic linear GBCAs Omniscan and Optimark (0.0809 % and 0.0204 % of the initial dose). Lower

gadolinium levels were measured in animals treated with the ionic linear GBCAs Magnevist (0.0095 % of initial dose) and Multihance (0.0016 % of the initial dose). Following the treatment with the macrocyclic compounds Gadovist, Dotarem, and Prohance, the gadolinium values in skin tissue were back in the same range as observed for the control animals from about day 24 post-treatment onwards. It should be noted that small amounts of gadolinium which were detected in the skin of control animals most probably resulted from a slight cross-contamination.

The observed gadolinium, which was retained for a long period in the skin, suggests that the gadolinium is existent in a water un-soluble state. The gadolinium, most likely released from the chelate, forms water insoluble particles, which then may be retained long-term in skin tissue.

Conclusions: Although all treatment groups received the same doses of contrast agents, differences in the exposure to contrast agents were observed. This difference in exposure was quantitatively correlated to the stability of the respective contrast agents. For example, the administration of a specific dose of Omniscan lead to a more than four times higher exposure to Gd as compared to the administration of the same dose of Magnevist.

Most of the administered Gd was eliminated from the skin within a time period of about two months. However, the repeated administration of linear GBCAs resulted in long-term retention of a small portion of the administered Gd in skin tissue of rats, with substantially higher values after treatment with non-ionic linear than after treatment with ionic linear GBCAs. Following treatment with macrocyclic GBCAs, Gd values in the skin were in the same range as observed in the controls from day 24 post injection onwards.

7.6 Potential long-time retention of Gadolinium in renally impaired rats (5/6 nephrectomized) after intravenous administration of Gadolinium based contrast agents

Published in: H Pietsch et al. [90]

Study objectives: The aim of this study was to determine the impact of a prolonged circulation time of Gadolinium based contrast agents (GBCAs) caused by reduced renal clearance on the Gd concentration and the long-term retention of gadolinium in the skin of rats after administration of different GBCAs.

An additional objective was to evaluate 5/6 nephrectomized rats as an animal model for prolonged circulation time of GBCAs as seen in renal impaired patients.

Study description: Renally impaired Han Wistar Rats (5/6-nephrectomized rats) were injected with Omniscan, Optimark, Magnevist or Gadovist. The contrast agents were administered once daily for five consecutive days into the tail vein at a dose of 2.5 mmol Gd/kg BW. Skin biopsies were taken at various time points, and the Gd concentration was determined by Inductive Coupled Plasma Mass Spectrometry over an observation period of 168 days post injection (p.i.).

Results: A greatly prolonged Gd presence in serum was observed in the 5/6-nephrectomized rats as compared with non-nephrectomized control rats. A single i.v. injection of 2.5mmol Gd /kg b.w. in 5/6 nephrectomized rats caused a similar exposure as seen in patients with sever renal impairments. The exposure and the plasma half-live in 5/6 nephrectomized rats were prolonged by a factor of 3 compared to healthy rats.

Differences in the skin Gd concentrations were observed between the four investigated GBCAs. For the non-ionic linear compounds, Omniscan and Optimark, high Gd concentrations were maintained in the skin over the observation period of up to 168 days p.i. For the ionic linear compound, Magnevist, comparatively lower Gd retention in the skin was observed over time. For the macrocyclic compound, Gadovist, the Gd values in the skin were even lower, and significantly lower than Gd values in the skin in Omniscan and Optimark treated animals. Overall, in 5/6 nephrectomized animals the Gd values in skin tissue of rats

after the treatment with linear GBCAs were at all investigated time points significantly higher than the values observed in the previous study with non-nephrectomized rats.

In 3 of the 12 Omniscan treated animals NSF-like skin lesions were observed.

Conclusion: The results of this nonclinical study support the use of 5/6-nephrectomized rats as a model for prolonged circulation time of GBCAs as seen in patients with severe renal impairment. Surgically-induced severe renal impairment resulted in delayed clearance of the administered GBCAs in the study animals. However, it should be kept in mind that 5/6 nephrectomized rats only reflect in same aspects like the GFR the pathology of renal impaired patients, eg, increased serum phosphate level does not occur in 5/6 nephrectomized rats, which is one of the main characteristics of patients with severe renal impairments.

The highest amount of Gd was observed in the skin after treatment with the non-ionic linear GBCAs, whereas the lowest Gd values were observed after treatment with the macrocyclic agent. This suggests that the difference in the Gd values observed in rat skin tissue after treatment with the different GBCAs is correlated to the stability of the different GBCAs and correspondingly to a different propensity of the different GBCAs to release Gd in vivo. However, the analytical method used does not distinguish between chelated and unchelated Gd.

The data demonstrate that the exposure to Gd is not only determined by the dose administered to an individual animal, but also by the complex stability of the respective compound and by the pharmacokinetics of the compound in the animal. A higher stability of a compound may reduce the propensity to release of Gd ions from the ligand in vivo and can thereby reduce the overall exposure to Gd.

7.7 Stability of Gadolinium-based contrast agents in human serum

Published in: T Frenzel et al. [73]

Study objectives: In order to assess the complex stability and Gd^{3+} dissociation rate of all marketed Gd-based MRI contrast agents (GBCA) assays in human serum at pH 7.4 and 37°C were performed.

Study description: The kinetic profiles of Gd^{3+} dissociation of GBCAs were determined by incubation for 15 days in human serum from healthy volunteers at a concentration of 1 mmol/L, pH 7.4 and 37°C. The initial rates of Gd^{3+} release and the amounts of Gd^{3+} released after 15 days were established by HPLC-ICP-MS analysis. In an attempt to simulate the situation in patients with end-stage renal disease who often have elevated serum phosphate levels, the influence of 10 mmol/L phosphate on Gd^{3+} dissociation was also investigated.

Results: The GBCAs were grouped according to their stabilities in native human serum at pH 7.4 and 37°C. The following tables summarize the release of Gd^{3+} after 15 days and initial Gd^{3+} release rate together with the respective 95 % confidence intervals in brackets.

Text Table 18: Gd^{3+}-release from non-ionic linear GBCAs after 15 days of incubation

	Omniscan	Optimark	Gadodiamide	Gadoversetamide
Gd^{3+} release after 15 d [%]	20 [17; 20]	21 [19; 22]	25 [22; 26]	29 [26; 32]
Initial Gd^{3+} release rate [%/d]	0.16 [0.15; 0.17]	0.44 [0.40; 0.51]	24 [12; 31]	17 [12; 30]

The rates of Gd^{3+} release from Omniscan and Optimark increased after 2-3 days to 2.4 [1.6; 2.9] %/d and 2.4 [1.9; 3.0] %/d respectively.

Text Table19: Gd^{3+}-release from ionic linear GBCAs after 15 days of incubation

	Magnevist	Multihance	Eovist	Ablavar
Gd3+ release after 15 d [%]	1.9 [1.2; 2.0]	1.9 [1.3; 2.1]	1.1 [0.76; 1.2]	1.8 [1.4; 1.9]
Initial Gd3+ release rate [%/d]	0.16 [0.12; 0.36]	0.18 [0.13; 0.38]	0.07 [0.05; 0.08]	0.12 [0.11; 0.18]

The rates of Gd^{3+} release from the ionic linear GBCAs remained almost constant during the 15 day incubation period.

Text Table 20: Gd^{3+}-release from macrocyclic GBCAs after 15 days of incubation

	Gadovist®	Prohance®	Dotarem®
Gd3+ release after 15 d [%]	< 0.1	< 0.1	< 0.1
Initial Gd3+ release rate [%/d]	< 0.007	< 0.007	< 0.007

No (limit of quantification was 0.1 %) Gd^{3+} release was observed from the three macrocyclic GBCAs.

In the presence of additional 10 mmol/L phosphate in serum the initial Gd^{3+} release rates of the non-ionic linear GBCAs, Omniscan and Optimark, increased about 100-fold, and, after 15 days, the amount of Gd^{3+} released from these agents was more than 75 % higher than in native serum. The initial rates found for the ionic linear GBCAs, increased about 12-30-fold, but, despite this increase in the initial rate, the amount of Gd^{3+} released after 15 days was comparable to that in native serum. The elevated phosphate level did not lead to any measurable release of Gd^{3+} from the three macrocyclic GBCAs.

Conclusion: The release of Gd^{3+} from all linear Gd complexes in human serum was several orders of magnitude greater than predicted by their conditional stability constants. After 15 days release of Gd^{3+} from the non-ionic linear GBCAs was about 10 times higher than from the ionic linear GBCAs. Elevated serum phosphate levels accelerated the release of

Gd^{3+} from non-ionic linear GBCAs and, to a lesser degree, from the ionic linear GBCAs. All three macrocyclic GBCAs remained stable in human serum at both normal and elevated phosphate levels.

7.8 The involvement of pro-inflammatory cytokines in nephrogenic systemic fibrosis: a systemic toxicity study in rats (M) with daily i.v. administration of gadodiamide (ZK 117439) over periods of 1 to 8 days to investigate the pathomechanism of skin lesions

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Study objectives: To further examine the potential mechanism of the skin changes observed in rats after administration of gadodiamide a nonclinical study was performed where gadodiamide was administered over various time-points to rats. Besides analyses of conventional toxicological parameters, additional technologies, including Gd determination in several tissues, multiplexed determination of serum cytokines and peptides, NMR-based metabolic profiling, immunohistochemistry, electron microscopy (including electron dispersive X-ray analysis for element-specific detection), and gene expression profiling were applied.

Study description: Three groups of 7 male Wistar [Hsd CpD:WU] rats received a daily intravenous injection of gadodiamide via the tail vein at a dose 2.5 mmol/kg. The first group was treated once, followed by necropsy 6 h post injection. The second group was treated for three days and underwent necropsy on day 4. A third group was treated for 8 days followed by necropsy on day 8. Each treatment group was paralleled by a control group of 7 male rats treated with 0.025 M $CaCl_2$ in 0.9 % NaCl (w/v). $CaCl_2$ was added to the vehicle in order to account for the high $CaCl_2$ content in the gadodiamide formulation. The injection volume of the gadodiamide and the control solution was set to 5 mL/kg.

One further group of 9 male rats received a single i.v. dose of 2.5 mmol/kg gadodiamide, with serum obtained at 2, 20 min and 1, 2, 6 and 24 h for toxicokinetic analysis. The serum samples were analyzed for Gd content with inductive-coupled plasma – optical emission

spectrometry (ICP-OES). (This method determines the content of Gd but is not able to differentiate between chelated, precipitated or free Gd ions.)

The effects of gadodiamide administration were assessed on the basis of clinical parameters (mortality, general observations, food and water consumption, body weight), as well as investigations in hematology, biochemical parameters and urinalysis. After sacrifice the animals were inspected macroscopically, organ weights were determined and histopathological examination was performed for major organs and tissues. Besides conventional H/E stain for the microscopic slides von Kossa stain was applied to skin and kidneys tissue in order to detect mineralization (calcium/gadolinium deposits).

In addition to the above-mentioned conventional analyses, samples from skin and whole blood were taken for gene expression analysis, Gd content was determined in skin, liver and femur; inductive-coupled plasma - atomic emission spectroscopy (ICP-AES), metabolic profiling was performed in urine (pre-values and day 5) and terminal serum samples based on nuclear magnetic resonance (NMR) measurement. Cytokines and serum peptides were determined in terminal plasma of all necropsy time points with a multiplexed fluorescent bead technology. Immunohistochemistry for smooth muscle actin (myofibroblasts), factor XIIIa (dermal dendrocytes), CD34 and collagen (circulating fibrocytes), CD1a/b (dermal Langerhans cells), tumor growth factor, osteopontin, CD3 (T-cells), 68-IB-3 (B-cells) and ED1-1 (macrophages) was applied to skin samples to identify and characterize dermal infiltrates (evaluation of the results of the immunohistochemistry part of the study is still ongoing). Furthermore skin samples of macroscopically affected and unaffected areas were processed for electron microscopy. Specific regions of interest with electron-dense material were further analyzed by electron dispersive X-ray (EDX) analysis which allows an element-specific detection of gadolinium in the micrographs.

The in-life phase, necropsy and the analysis of the conventional toxicological parameters of the study were performed under GLP. The processing and determination of all additional parameters were undertaken in functions or institutions not working under GLP rules.

Results: at the tested dose of 2.5 mmol/kg gadodiamide the following observations were made at the different timepoints (observations are recorded only for the day of their first appearance)**:**

Clinical & laboratory parameters:

Day 1 (6h):

- increase in blood monocytes
- transient increase in serum calcium and AST, slight decrease in the albumin fraction
- slight tubular vacuolation in the kidneys (marked on day 8)
- significant increase in 13 cytokines/serum peptides involved in control of vascular permeability and inflammatory processes (interleukin 1α, 7, 10, inducible protein 10, lymphotactin, monocyte chemoattractant proteins [MCP], macrophage inflammatory proteins [MIP], TNF-α, tissue inhibitor of matrix metalloproteinase, osteopontin, vascular endothelial growth factor [VEGF])

Day 3 or 4 (necropsy):

- slight increase in ALT, decrease in alkaline phosphotase (ALP), slight decrease in glucose and increase in cholesterol
- decrease in food consumption and body weight gain, slight body weight loss (animals of necropsy group day 3)
- changes in cytokines/serum peptides as described for day 1 with additional significant increase of 3 cytokines/serum peptides (haptoglobin, myeloperoxidase, lipocalin-2)

Day 7 or 8:

- slight swelling of the head at the end of the observation period
- hematuria identified during clinical observation in one animal and confirmed by urinanalysis for several animals
- decrease in food consumption associated with a clear decrease in body weight gain partially with body weight loss (-15 %). Clear signs of impaired condition such as emaciation and ruffled fur were noted for these animals at the end of the observation period
- decrease of the absolute absolute and relative thymus weight (-36 % and -26%, respectively) and the spleen (-19 % and -5%, respectively). In the kidneys, an increase of the relative organ weight was noted (+15 %)
- slight increase in monocyte, neutrophil, eosinophil, basophil counts and large unstained cell number

- increase in relative alpha- and beta-globulin fraction accompanied with an decrease in the albumin fraction (-16 %)

In summary, the laboratory investigations revealed signs of an inflammatory response in the study animals on day 7 indicated by the increase in white blood cell parameters as well as a shift in the globulin fractions (mainly beta-globulin). The increase in monocytes in the study animals already observed on day 1 and day 4 indicates the onset of the inflammatory reaction. This is further supported by the increase in serum concentrations of several pro-inflammatory cytokines and peptides which are involved in Ca homoeostasis, monocyte/macrophage activation and the regulation of vascular permeability. The time course of these markers displays an inverse bell-shaped pattern, ie, the markers show a sharp increase on day 1, a lower increase on day 4 and again a higher increase at day 8.

The increased amount of urinary blood in the study animals at day 7/8 most likely reflects an impairment of the kidney due to the daily administration of a high dose of a contrast medium eliminated via the urinary pathway which is also evidenced in an increase of the relative kidney weight and the microscopic observation of marked tubular vacuolation.

Impaired kidney function and general health status were also indicated in the urinary metabolic profile on day 5 through increased excretion of creatine, glucose and certain amino acids and decreased excretion of citrate and ketone bodies. Serum metabolic profiling of day 8 also reflected the bad health status (increased concentrations of creatine, glucose and taurine) and showed evidence for inflammatory reactions (increased amount of NO-precursors).

Gadolinium values in serum:

Serum analysis for Gd showed rapid distribution and an almost quantitative excretion within 24 h. Traces of Gd were found in liver, femur and skin in study animals after the first administration, increasing with the number of injections.

Histopathology assessment of the skin:

Day 3 or 4 (necropsy):

- alterations of the skin such as scab formation at several locations (back, hind limbs, flanks and abdomen), skin reddening, swelling and skin fissures at the flanks (6 of 7 animals). Skin lesions microscopically evident with ulceration, crust (2 of 7 animals), dermal inflammatory infiltrations and positive von Kossa stain.

Day 7 or 8:

- additionally to the skin findings of day 3, which were observed at higher incidence microscopically acanthosis and interface dermatitis was noted; macroscopic skin lesions observed in all animals.

Electron microscopy of skin tissue:

Electron microscopy of skin samples from day 8 revealed an increased number of macrophages, epidermal ulcerations and thickened collagen fibers in the middle and deeper layer of the dermis of the study animals. Electron-dense particles were found in the macrophages and adjacent to or within the fibers. Electron dispersive X-ray analysis confirmed the existence of Gd in the macrophages but not in the collagen fibers.

Conclusions: Daily intravenous gadodiamide administration in rats at a dose of 2.5 mmol/kg resulted in macroscopic skin lesions already after three administrations. The lesions were accompanied by changes in clinical chemistry and hematology pointing towards inflammatory processes. Gd was detectable in skin and other tissues of the study animals after the first administration.

Additionally, time-dependent changes in kidneys and impaired general condition were observed in the study animals: 6 hours after the first administration vacuolation in the tubuli

of the kidneys was noted. At the end of the study, the animals were emaciated and incidence and severity of skin and kidney lesions were increased.

The significant increases in serum concentration of certain cytokines or serum peptides observed already 6 h after the first administration appear to support a rapid influence of injected gadodiamide on these mediators of inflammatory processes which precedes the skin lesions in the study animals.

7.9 Summary of nonclinical results obtained in mechanistic studies to elucidate te pathomechanism

Nonclinical in-vivo studies in rats were initiated by BSP to investigate the possible association of NSF and GBCAs in more detail. The nonclinical studies were designed to provide additional information on this possible association and to evaluate the potential effects of the stability differences between the GBCAs. Some variability of results in different settings cannot be excluded. Also, caution should be used in extrapolating these nonclinical findings to humans.

The results of the above listed nonclinical studies can be summarized as follows:

- There is a possible association between the administration of certain formulations of gadolinium-based contrast agents and the induction of NSF-like skin lesions in a rat animal model.

- Some rats treated with non-ionic Gd-based substances (gadodiamide, gadoversetamide, Gd-EDTA) in certain studies developed NSF-like skin changes, including ulceration, fibrosis, increased cellularity and CD34 positive immunostaining. Therefore the term “NSF-like lesions” was applied to the findings in the rat studies.

- The most pronounced effects on skin were observed in animals following the administration of gadodiamide, the drug substance of Omniscan without excess of free

ligand, and gadoversetamide, the drug substance of Optimark without excess of free ligand.

- Following the administration of marketed Gd-based contrast agents, NSF-like skin changes (both macroscopic and microscopic) were observed in some Omniscan-treated animals. No NSF-like effects (neither macroscopic nor microscopic) were observed in any of the animals treated with Optimark, Magnevist, Multihance, Eovist, Gadovist, Dotarem, and Ablavar.

- No effects on the skin were observed after treatment with Gd-free ligands (eg, Ca-EDTA and caldiamide) and saline.

- No relevant effect of Zn supplementation (subcutaneous [s.c.] or per oral [p.o]) on occurrence of skin changes was detected. No relevant changes in serum / tissue concentrations of endogenous ions such as Zn and Cu were detectable after administration of the various tested compounds. Thus there is no evidence from these nonclinical studies that NSF-like skin lesions are elicited by Zn depletion caused by excess ligand contained in the formulation of GBCAs.

- The skin lesions observed in the animal studies (macroscopically and microscopically) were correlated with high Gd concentrations determined in the skin, and not with a depletion of endogenous ions.

- Among the marketed Gd-based contrast agents evaluated in the nonclinical studies, the highest Gd concentration in the skin was observed after the treatment with Omniscan. Further, higher Gd concentrations were correlated with the histopathological skin lesions in these animals. Lower Gd concentrations were obtained in Optimark-treated animals compared to Omniscan treated animals, but the Optimark values were still considerably higher than the values obtained after treatment with the other marketed products evaluated in this study.

- Presence of excess ligand in the formulation of less stable GBCAs (eg, gadodiamide, gadoversetamide) reduced the Gd concentrations measured in skin tissue samples.

- Different in-vitro stabilities of the various GBCAs appear to correlate with different concentrations of Gd in the skin.

- Most of the administered Gd was eliminated from the skin within a time period of about two months. However the repeated administration of linear GBCAs resulted in long-term retention of a small portion of the administered Gd in skin tissue of rats, with substantially higher values after treatment with non-ionic linear than after treatment with ionic linear GBCAs. Following treatment with macrocyclic GBCAs, Gd values in the skin were in the same range as observed in the controls from day 24 post injection onwards.

- In 5/6 nephrectomized rats high Gd concentrations were maintained in the skin after treatment with the non-ionic linear compounds, Omniscan and Optimark, over the observation period of up to 168 days p.i. for Magnevist, comparatively lower Gd retention in the skin was observed. At all investigated time points the Gd values after the treatment with linear GBCAs were significantly higher than the values observed in the previous study with non-nephrectomized rats.

- The differences in long-term Gd retention are qualitatively correlated to the stability of the respective GBCAs.

- Increases in serum concentration of certain cytokines or serum peptides observed 6 h after the first administration of gadodiamide would appear to support a rapid influence of this compound on these mediators of inflammatory processes as a possible precursor to the appearance of skin lesions.

In summary, in rat studies designed in an effort to simulate the exposure of ESRD patients to GBCAs, skin lesions were observed after administration of non-ionic linear GBCAs, which were similar to skin lesions in NSF patients and which correlated with the detection of high

Gd-levels in skin tissue. Development of these lesions was preceded by elevated serum cytokines which may play a role in fibrosis and in the recruitment of both monocytes and macrophages.

Furthermore, the nonclinical studies demonstrated that the presence of excess ligand alone did not appear to influence lesion formation. In addition, the excess ligand present in the least stable GBCAs (Omniscan and Optimark) reduced skin Gd concentrations.

8. Overall Summary and Conclusions

8.1 Magnevist

NSF is a very rare disease that, thus far, has predominantly been observed in patients with severe renal impairment. The etiology of NSF is still unknown but is thought to be multifactorial. The particular combination and severity of co-factors necessary to trigger the development of NSF has not, as yet, been elucidated. Exposure to Gd-based contrast agents (GBCAs) has been identified as a potential risk factor for acquiring this serious and disabling disease. This theory was first proposed in 2006. A number of other mechanisms and potential risk factors have also previously been proposed, including surgery and/or the occurrence of thrombosis or other vascular injury, proinflammatory state, and the administration of high doses of erythropoietin. Of note, a number of NSF cases have been reported in the absence of documented GBCA exposure.

In order to evaluate whether there are differences among the various GBCAs regarding their possible likelihood to trigger NSF-like symptoms in at risk patients, many factors should be considered. These include the available clinical evidence for each of the GBCAs, taking into account the number of reports, published studies, range of approved indications, range of dosages approved for use in CE-MRI, the number of administrations, and the length of time since initial approval. In addition, based on the prevailing theory on the possible role of GBCAs in the development of NSF, the following factors should also be considered:

- Complex stability of GBCAs;

- Pharmacokinetics of GBCAs;
- Results of nonclinical exploratory studies intended to evaluate possible differences between GBCAs regarding their potential risk to trigger NSF-like skin changes.

Since its approval in 1988 in the US and in a number of other countries as the first GBCA for use in MRI, Magnevist, with more than 100 million doses administered thus far, has the most administrations and the longest clinical experience among all marketed GBCAs. In the US, where nearly half of the total administrations have taken place, Magnevist has the broadest range of indications of all marketed GBCAs and is the only GBCA that is approved and indicated for all of the following types of MR imaging: CNS, extracranial-extraspinal tissues (head and neck) and body (excluding the heart) in both adult and pediatric populations above 2 years of age.

In July 2006 the company received its first report of a patient who, according to the report, had developed NSF following Magnevist administration. From receipt of that first report until the present, the majority of reports claiming that NSF or NSF-like symptoms developed following Magnevist administration have come from lawsuits filed against Bayer, and often against some or all of the other GBCA manufacturers. Many of these reports contain only minimal information.

Of the 554 case reports received and evaluated to date, 233 contain no documented administration of Magnevist. In the absence of any evidence substantiating Magnevist administration, these reports are excluded from further analysis pending receipt of additional information. In the remaining 321 cases, patients were reported to have received Magnevist alone or in combination with other products. 142 of these reports were confounded by the administration of other GBCAs in the same timeframe, in which NSF could plausibly have developed. In the remaining 179 reports (55.8%), Magnevist was the only product reported. In 98 of these reports the association to Magnevist is considered possible, based primarily on a temporal association (18 months or less) between documented and generally unconfounded Magnevist administration and onset of symptoms, lack of a plausible alternative explanation,

and confirmation of a diagnosis of NSF via skin biopsy or other means (e.g. a clinical diagnosis based on patient history and symptom presentation).

Published studies in the medical literature suggest the incidence rates of NSF following the administration of Magnevist to be lower than that of non-ionic linear GBCAs, ie, Omniscan. The FDA has requested the sponsors of all marketed GBCAs to conduct post-marketing studies to assess the magnitude of the potential risk for the development of NSF in patients with moderate to severe renal impairment. Bayer has initiated such a study with Magnevist ("MRI study"), which is currently ongoing in the US.

Magnevist has demonstrated a well established and favorable efficacy and safety profile both in clinical trials and during the post-marketing surveillance period. It is predominantly eliminated via the kidneys without any biotransformation or decomposition in vivo. In vitro results indicate that Magnevist does not bind to human plasma protein. Magnevist can be removed from the body by hemodialysis.

Based on available information on complex stability of the various Gd-chelates, it can be concluded that the non-ionic linear chelates (eg, Omniscan, Optimark) have a higher propensity to release Gd^{3+} than either the ionic-linear chelates (eg, Magnevist, Multihance) or the macrocyclic chelates (Dotarem, Gadovist, Prohance). Furthermore, available information indicates that the likelihood of $Gd3^{+}$ release with Magnevist does not differ from that of other ionic-linear Gd-chelates (including Multihance).

Nonclinical study results suggest that deposition of Gd in the skin and the development of skin lesions is correlated with extended exposure to Gd-based contrast agents and with the complex stability of the Gd-chelate complexes and their propensity to release Gd3+ ions. However, due to the analytical difficulties to distinguish chelated from unchelated Gd in the animal studies the company has undertaken, the hypothesis regarding a potential role of complex stability cannot be definitively confirmed. Furthermore, some of the nonclinical findings suggest that Gd may play a role in the upregulation of inflammatory cytokines that appears to proceed disease onset. Based on available clinical evidence and, nonclinical study

results - and taking into consideration the particular GBCA's properties regarding complex stability and its pharmacokinetics in humans - the potential likelihood of a particular Gd-chelate to release Gd3+ ions seems to depend on the chelate's physicochemical properties and might be increased in case the elimination of the Gd-chelate from the body is reduced. The potential for Gd dissociation appears higher after exposure to non-ionic linear agents such as Omniscan and Optimark than compared to all other ionic-linear Gd-based contrast agents, including Magnevist; the lowest potential for Gd dissociation appears to be with macrocyclic GBCAs.

In nonclinical rat studies designed in an effort to simulate the exposure of ESRD patients to GBCAs, skin lesions were observed in some animals after administration of non-ionic linear GBCAs, which were similar in many aspects to skin lesions in NSF patients and which correlated with the detection of high Gd-levels in skin tissue. Development of these lesions was preceded by elevated serum cytokines which may play a role in fibrosis and in the recruitment of both monocytes and macrophages. The nonclinical studies demonstrated that following the administration of marketed GBCAs, NSF-like skin changes (both macroscopic and microscopic) were observed in some Omniscan-treated animals. The skin lesions observed in these animals were correlated with high Gd concentrations determined in the skin, and not with a depletion of trace elements such as Zn. No NSF-like lesions (neither macroscopic nor microscopic) were observed in any of the animals treated with Optimark, Magnevist, Multihance, Primovist (Eovist), Gadovist, Dotarem, and Vasovist (Ablavar).

For all GBCAs investigated in the preclinical studies, most of the administered Gd was eliminated from the skin within a time period of about two months. However, the repeated administration of linear GBCAs resulted in long-term retention of a small portion of the administered Gd in skin tissue of rats, with substantially higher values after treatment with non-ionic linear than after treatment with ionic-linear GBCAs. Following treatment with macrocyclic GBCAs, Gd values in the skin were in the same range as observed in the control animals, which received no Gd, from day 24 post injection onwards.

In conclusion, of the above summarized nonclinical and clinical data, Bayer believes the risk potential of Magnevist associated with the development of NSF/ NSF-like symptoms in the at risk population to be lower than that of non-ionic linear GBCAs. If administered according to the approved indications and dose, Bayer believes there is no difference in risk potential associated with the development of NSF/NSF-like symptoms between Magnevist and any other ionic linear GBCA.

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